



Sal I Bam H I

----- - pUC 19

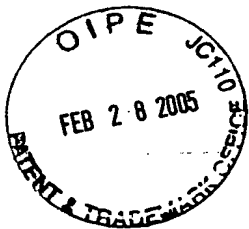
Xba I

1 ATGGAGTCAA AGTTTGCTCA CATCATTGTT TTCTTTCTTC TTGCAACTTC
original sequence - ag a
51 CTTTGAAACT CTCTTGGCAC GAAAAGAAAG Tgatggacca gagatcttag
mutagenic primer
101 aacTTCAAAA GGAATTTGAA TGCAATGGAA AACAAAGGTG GCCAGAACTT
151 ATTGGTGTAC CAACAAAGCT TGCTAAGGGG ATAATTGAGA AGGAAAATTC
201 ACTCATAACT AATGTTTCTG TACTACTGAA TGGTTCTCCA GTCACAATGG
251 ATTATCGTTG TAATCGAGTT CGTCTTTTGG ATAACATTTT GGGTGATGTT
301 GTACAAATTC CTAGGGTGGC TTAA

Figure 1

1 GAATTCCGCA AGGAgcacac ccggctgtcc acctgCTGCA GAGATGGTGC
upstream primer
51 ACGCAACCTC CCCGCTGCTG CTGCTGCTGC TGCTCAGCCT GGCTCTGGTg
cc t original sequence
101 gctccccgga tccctgccag AAAGTGCTCG CTGACTGGGA AATGGACCAA
mutagenic primer
151 CGATCTGGGC TCCAACATGA CCATCGGGGC TGTGAACAGC AGAGGTGAAT
201 TCACAGGCAC CTACATCACA GCCGTAACAG CCACATCAAA TGAGATCAAA
251 GAGTCACCAC TGCATGGGAC ACAAACACC ATCAACAAGA GGACCCAGCC
301 CACCTTTGGC TTCACCGTCA ATTGGAAGTT TTCAGAGTCC ACCACTGTCT
351 TCACGGGCCA GTGCTTCATA GACAGGAATG GGAAGGAGGT CCTGAAGACC
401 ATGTGGCTGC TGCAGTCAAG TGTTAATGAC ATTGGTGATG ACTGGAAAGC
451 TACCAGGGTC GGCATCAACA TCTTCACTCG CCTGCGCACA CAGAAGGAGT
501 GAGGATGGCC CCGCAAAGCC AGCAACAATG CCGGAGTGCT GACACTGCTT
↓ Hind III
551 GTGATATTCC TCCCAATAA AGCTTG

Figure 2



EcoR I
↓
1 GAATTTCGCAT ATGGCTGAAG CTGGTATCAC CGGTACTTGG TACAACCAGC
51 TGGGGTCTAC CTTTCATCGTT ACCGCTGGTG CTGACGGTGC ACTGACCGGT
101 ACTTACGAAA GCGCTGTTGG TAACGCTGAA AGCCGTTATG TTCTGACCGG
151 TCGTTACGAC TCTGCTCCGG CTACCGACGG TTCTGGTACT GCTCTGGGTT
201 GGACCGTTGC TTGAAAAAAC AACTACCGTA ACGCTCACTC TGCTACCACC
251 TGGTCTGGCC AGTACGTTGG TGGTGCTGAA GCTCGTATCA ACACCCAGTG
301 GCTGCTGACC TCTGGTACCA CCGAAGCTAA CGCTTGAAAA TCTACCCTGG
351 TTGGTCACGA CACGTTCCACC AAAGTTAAAC CGTCTGCTGC TTCTATCTAGA
↑
Xba I

Figure 3

Sal I altered Bam H I*

----- pUC 19

Xba I

1 ATGGATGTTT ACAAGGAAGT TAATTTTCGTT GCTTACCTAC TAATTGTTCT
51 TGGTAAGATT TTCCTTTACT CCTTTGTTTT AAAAAATAAA AAAACAAAAA
101 AAATCTTGGT TTATACATAT ATATACACAC AAGTAGTTTT ATTTTTTTCC
151 TTTATATTAT ATTGTTGTA GGAATATTTT TACTTGTTAG CGTGGTGGAA
201 CATGTTGATG CGAAGATCTG TACTAAAGAA TGTGGTAATC TTGGGTTTGG
251 GATATGCCCA CGTTCAGAAG GAAGTCCGAA AAATCCCATA TGCATCAATT
301 GTTGCTCAGG CTATAAGGGT TGTAATTATT ATAGTGTTTT CGGGAGATTT
351 ATTTGCGAAG GAGAATCTGA CCTAAAAAAC CCAAAAGCTT GCCCCCTAAA
401 TTGTGATACA AATATGTCCT ATTCAAGATG CCCCCATTCA GAAGGAAAAT
451 CGCTAATTTA TCCCACCGGA TGTACCACAT GTTGACACAGG GTACAAGGGT
501 TGCTACTATT TCGGTAAAAA TGGCAAGTTT GTATGCGAAG GAGAGAGTGA
551 TGAACCCAAG GCAAATATGT ACCCTGCAAT GTGA

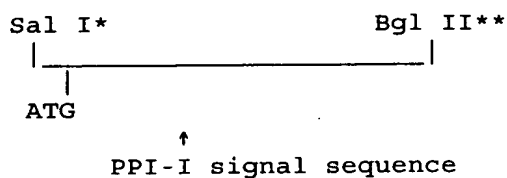
* result of PCR error during isolation of the PPI-II sequence

Figure 4

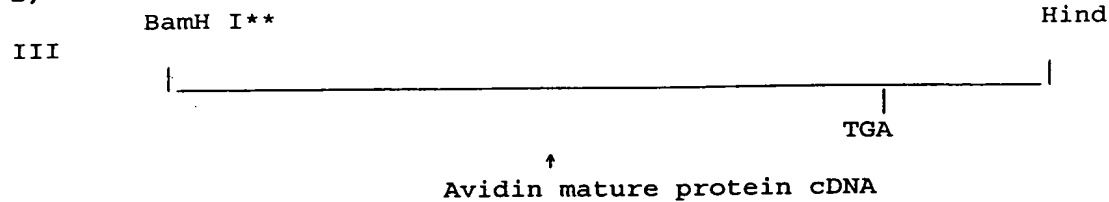


3/28

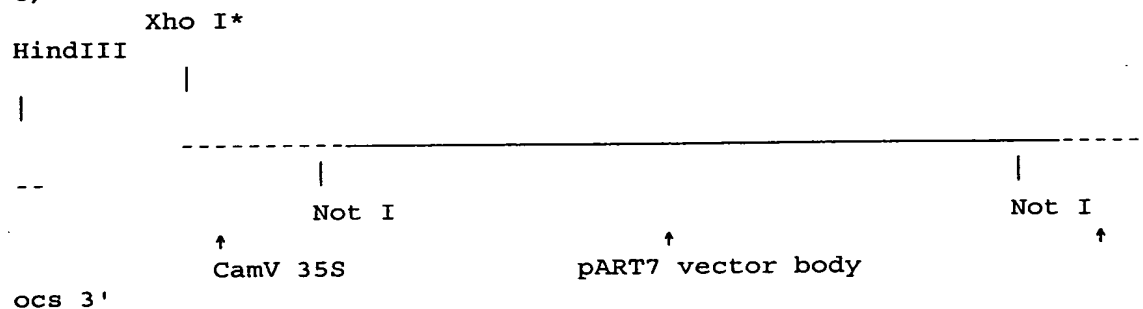
A)



B)



C)



* compatible cohesive ends
** compatible cohesive ends

Figure 5

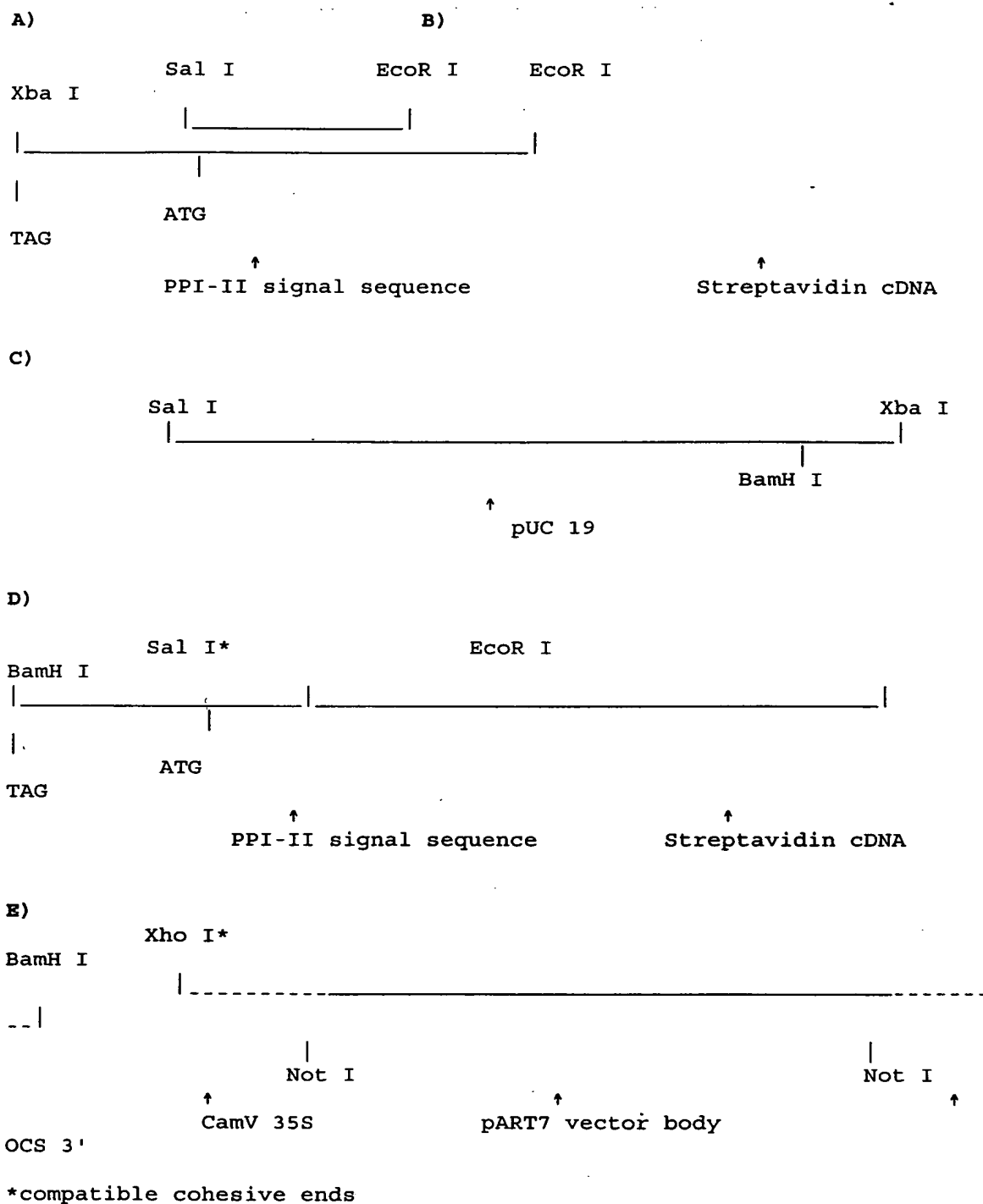
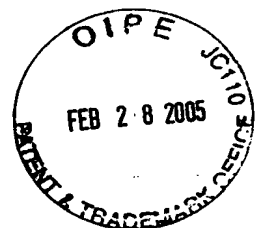
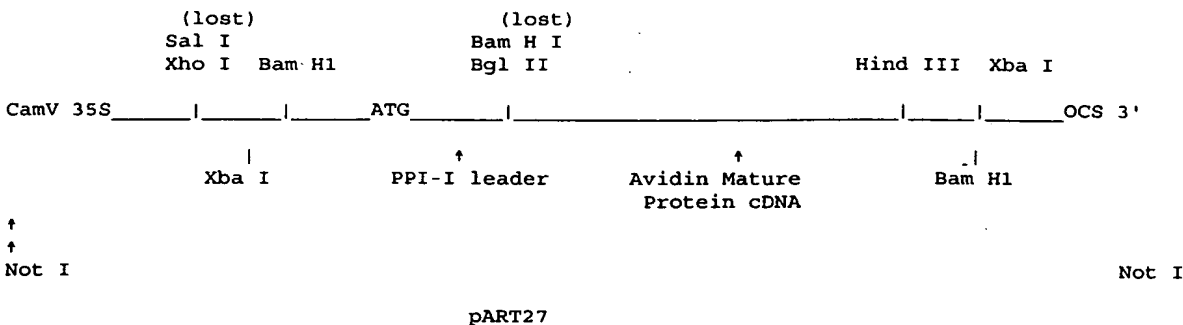


Figure 6



5/28

A)



B)

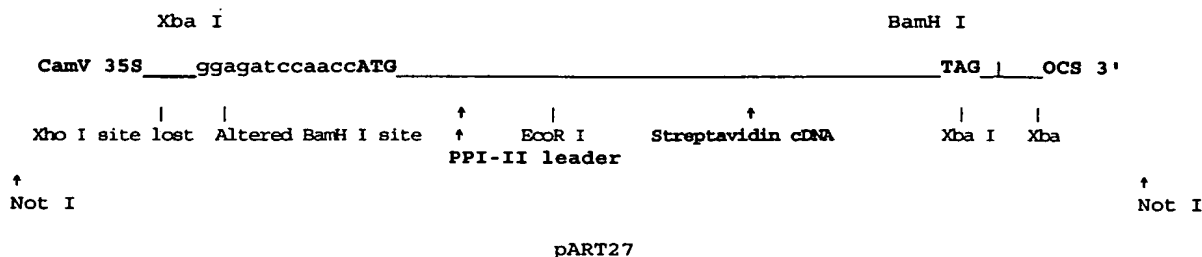


Figure 7

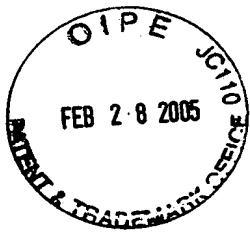
A)

1 ATGGAGTCAA AGTTTGCTCA CATCATTGTT TTCTTCTTC TTGCAACTCC
51 CTTTGAAACT CTCTTGGCAC GAAAAGAAAAG TGATGGACCA GAGATCCCTG
101 CCAGAAAGTG CTCGCTGACT GGGAAATGGA CCAACGATCT GGGCTCCAAC
151 ATGACCATCG GGGCTGTGAA CAGCAGAGGT GAATTCACAG GCACCTACAT
201 CACAGCCGTA ACAGCCACAT CAAATGAGAT CAAAGAGTCA CCATTGCATG
251 GGACACAAAA CACCATCAAC AAGAGGACCC AGCCCACCTT TGGCTTCACC
301 GTCAATTGGA AGTTTTTCAGA GTCCACCACT GTCTTCACGG GCCAGTGCTT
351 CATAGACAGG AATGGGAAGG AGGTCCTGAA GACCATGTGG CTGCTGCGGT
401 CAAGTGTTAA TGACATTGGT GATGACTGGA AAGCTACCAG GGTCGGCATC
451 AACATCTTCA CTCGCCTGCG CACACAGAAG GAGTGA

B)

cleavage site
↓
1 MESKFAHIIV FLLATPFET LLARKESDGP EipARKCSLT GKWTNDLGSN
51 MTIGAVNSRG EFTGTYITAV TATSNEIKES PLHGTQNTIN KRTQPTFGFT
101 VNWKFSSTT VFTGQCFIDR NGKEVLKTMW LLRSSVNDIG DDWKATRVGI
151 NIFTRLRTQK E*

Figure 8



6/28

A)

```
1  ATGGATGTTT ACAAGGAAGT TAATTTTCGTT GCTTACCTAC TAATTGTTCT
51  TGGTAAGATT TTCCTTTACT CCTTTGTTTTT AAAAAATAAA AAAACAAAAA
101 AAATCTTGGT TTATACATAT ATATACACAC AAGTAGTTTTT ATTTTTTTTCC
151 TTTATATTAT ATTTGTTGTA GGAATATTTT TACTTGTTAG CGTGGTGGA
201 CATGTTGATG CGAAGATCTG TACTAAGAAT TCGCATATGG CTGAAGCTGG
251 TATCACCGGT ACTTGGTACA ACCAGCTGGG GTCTACCTTC ATCGTTACCG
301 CTGGTGCTGA CGGTGCACTG ACCGGTACTT ACGAAAGCGC TGTTGGTAAC
351 GCTGAAAGCC GTTATGTTCT GACCGGTCGT TACGACTCTG CTCCGGCTAC
401 CGACGGTTCT GGTACTGCTC TGGGTTGGAC CGTTGCTTGG AAAAACAAC
451 ACCGTAACGC TCACTCTGCT ACCACCTGGT CTGGCCAGTA CGTTGGTGGT
501 GCTGAAGCTC GTATCAACAC CCAGTGGCTG CTGACCTCTG GTACCACCGA
551 AGCTAACGCT TGGAAATCTA CCCTGGTTGG TCACNACACG TTCACCAAAG
601 TTAAACCGTC TGCTGCTTCT ATCTAG
```

B)

cleavage site

```
1  MDVHKEVNFV AYLLIVLGIF LLVSVEHVD AKICTKnshM AEAGITGTWY
51  NQLGSTFIVT AGADGALTGT YESAVGNAES RYVLTGRYDS APATDGSGTA
101 LGWTVAWKNN YRNAHSATTW SGQYVGGAEA RINTQWLLTS GTTEANAWKS
151 TLVGHDTFTK VKPSAASI*
```

Figure 9

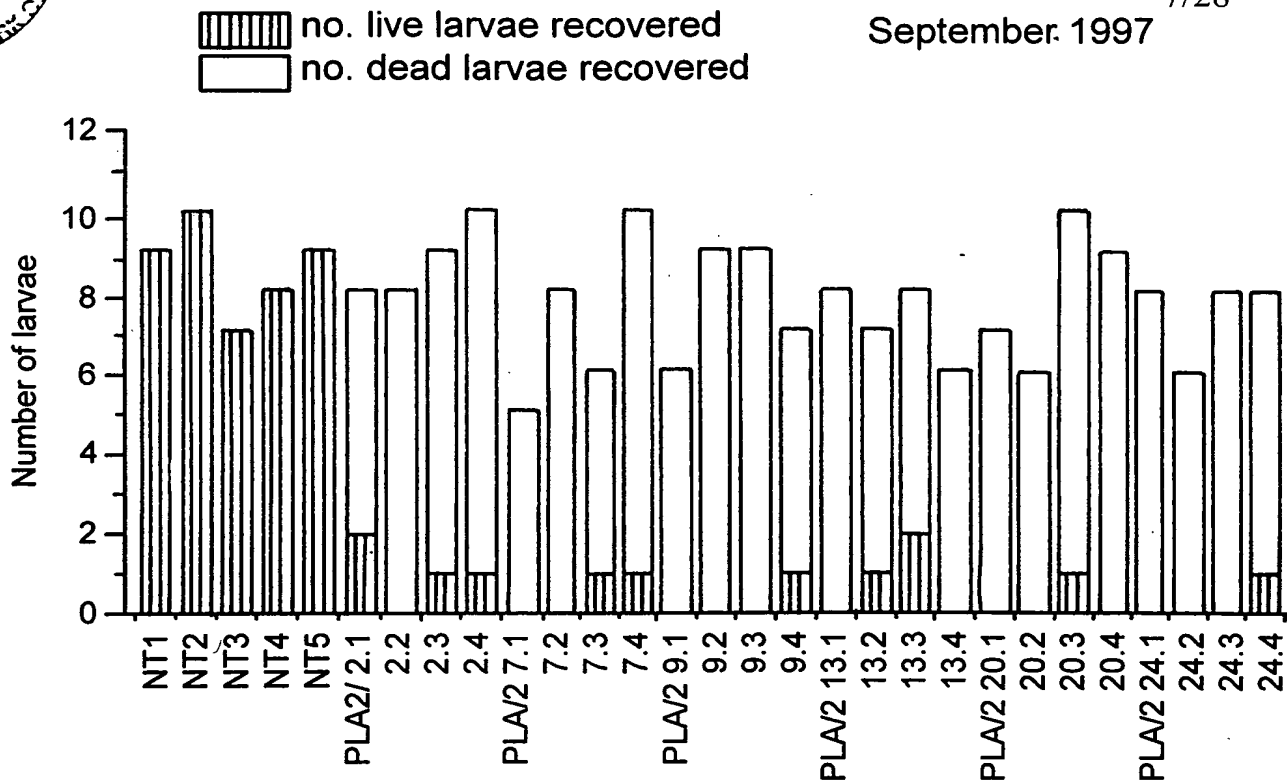


FIG. 10

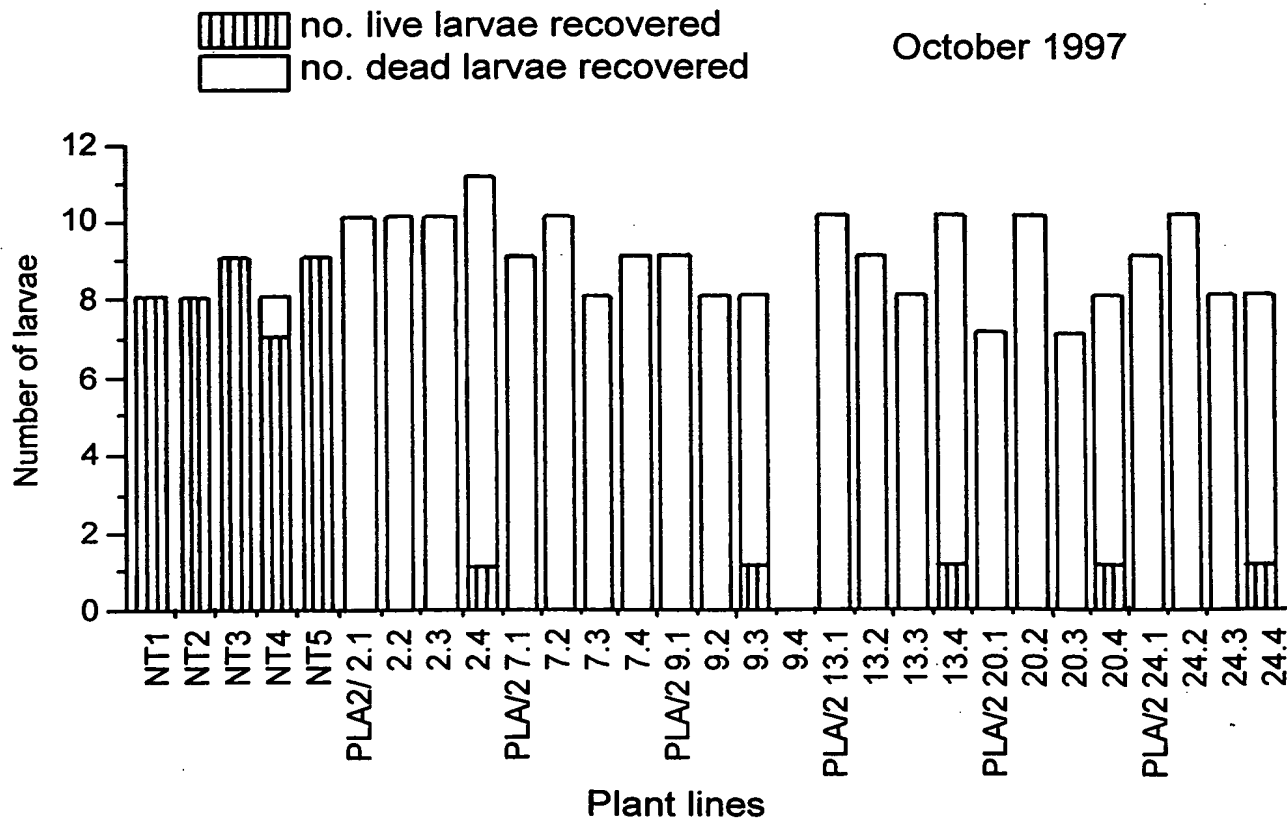
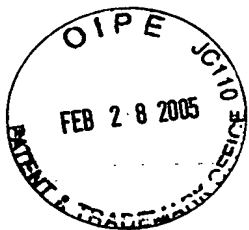


FIG. 11



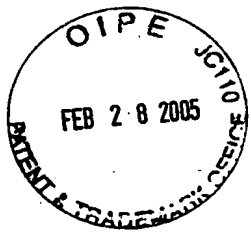
A)

1 CCCTCCGTCC CCGCCGGGCA ACAACTAGGG AGTATTTTTC GTGTCTCACA
51 TGC GCAAGAT CGTCGTTGCA GCCATCGCCG TTTCCCTGAC CACGGTCTCG
101 ATTACGGCCA GCGCTTCGGC AGACCCCTCC AAGGACTCGA AGGCCAGGT
151 CTCGGCCGCC GAGGCCGGCA TCACCGGCAC CTGGTACAAC CAGCTCGGCT
201 CGACCTTCAT CGTGACCGCG GGCGCCGACG GCGCCCTGAC CGGAACCTAC
251 GAGTCGGCCG TCGGCAACGC CGAGAGCCGC TACGTCCTGA CCGGTCGTTA
301 CGACAGCGCC CCGGCCACCG ACGGCAGCGG CACCGCCCTC GGTTGGACGG
351 TGGCCTGGAA GAATAACTAC CGCAACGCCC ACTCCGCGAC CACGTGGAGC
401 GGCCAGTACG TCGGCGGCGC CGAGGCGAGG ATCAACACCC AGTGGCTGCT
451 GACCTCCGGC ACCACCGAGG CCAACGCCTG GAAGTCCACG CTGGTCGGCC
501 ACGACACCTT CACCAAGGTG AAGCCGTCCG CCGCCTCCAT CGACGCGGCG
551 AAGAAGGCCG GCGTCAACAA CGGCAACCCG CTCGACGCCG TTCAGCAGTA
601 GTCGCGTCCC GGCACCGGCG GGTGCCGGGA CCTCGGCC

B)

1 MRKIVVAAIA VSLTTVSITA SASADPSKDS KAQVSAAEAG ITGTWYNQLG
51 STFIVTAGAD GALTGTYESA VGNAESRYVL TGRYDSAPAT DSGGTALGWT
101 VAWKNNYRNA HSATTWSGQY VGGAEARINT QWLLTSGTTE ANAWKSTLVG
151 HDTFTKVKPS AASIDAAKKA GVNNGNPLDA VQQ

Figure 12



9/28

BEST AVAILABLE COPY



Figure 13

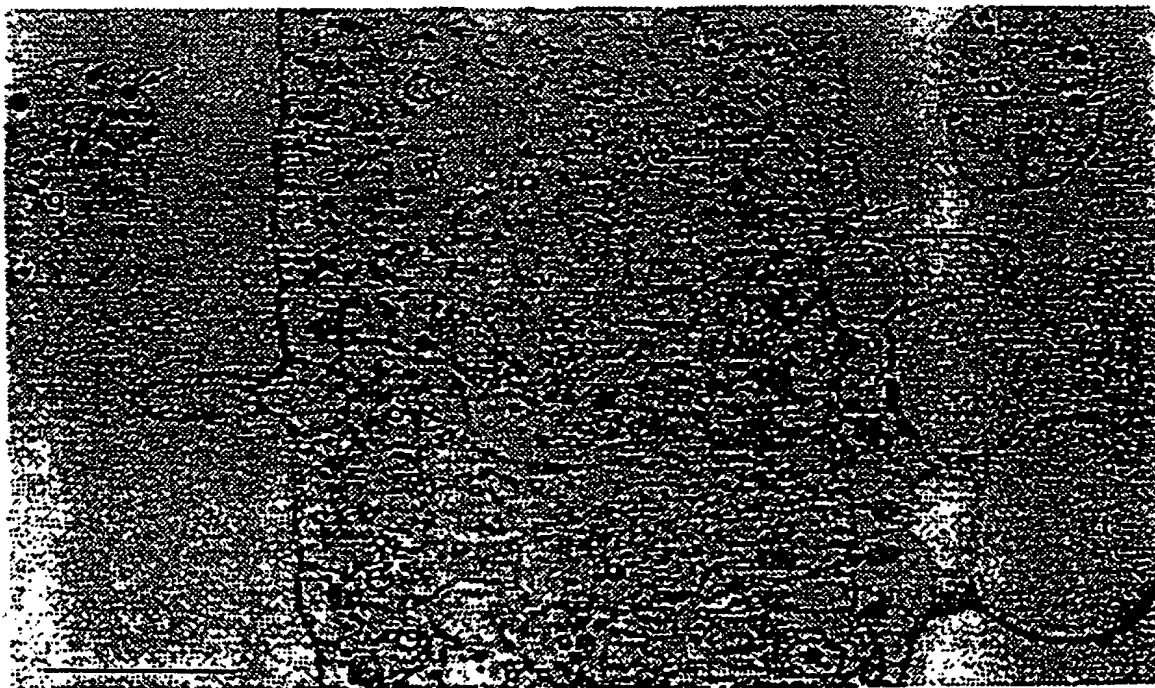
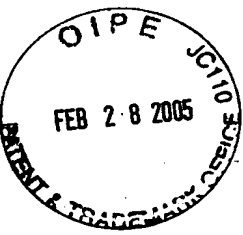


Figure 14



10/28

BEST AVAILABLE COPY

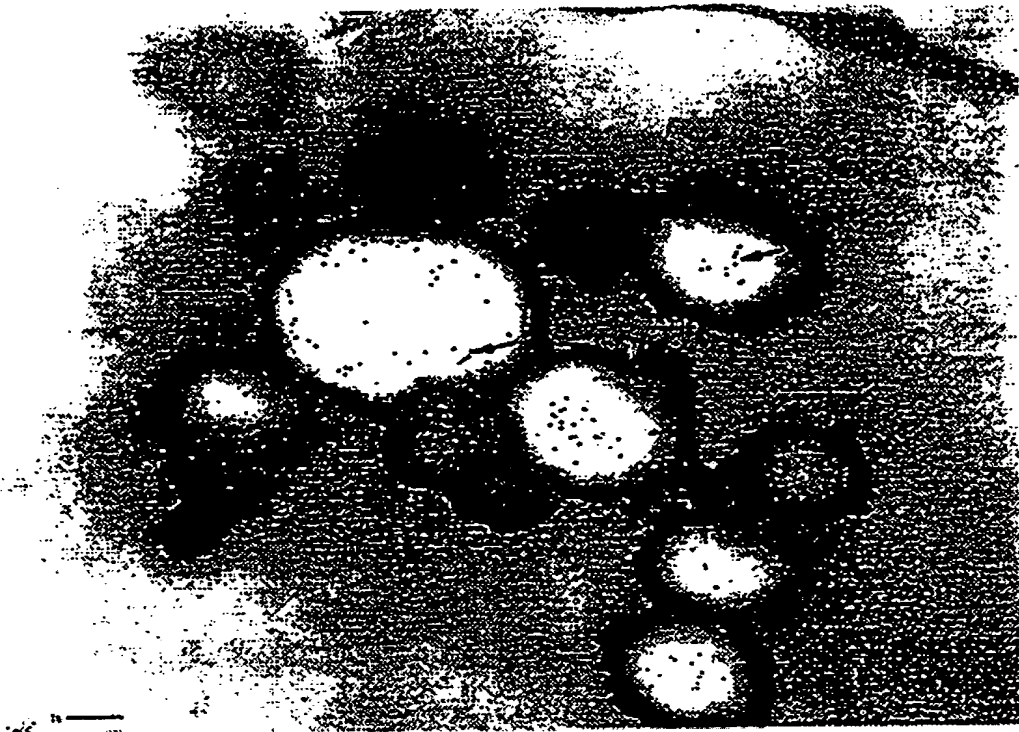


Figure 15



Figure 16



11/28

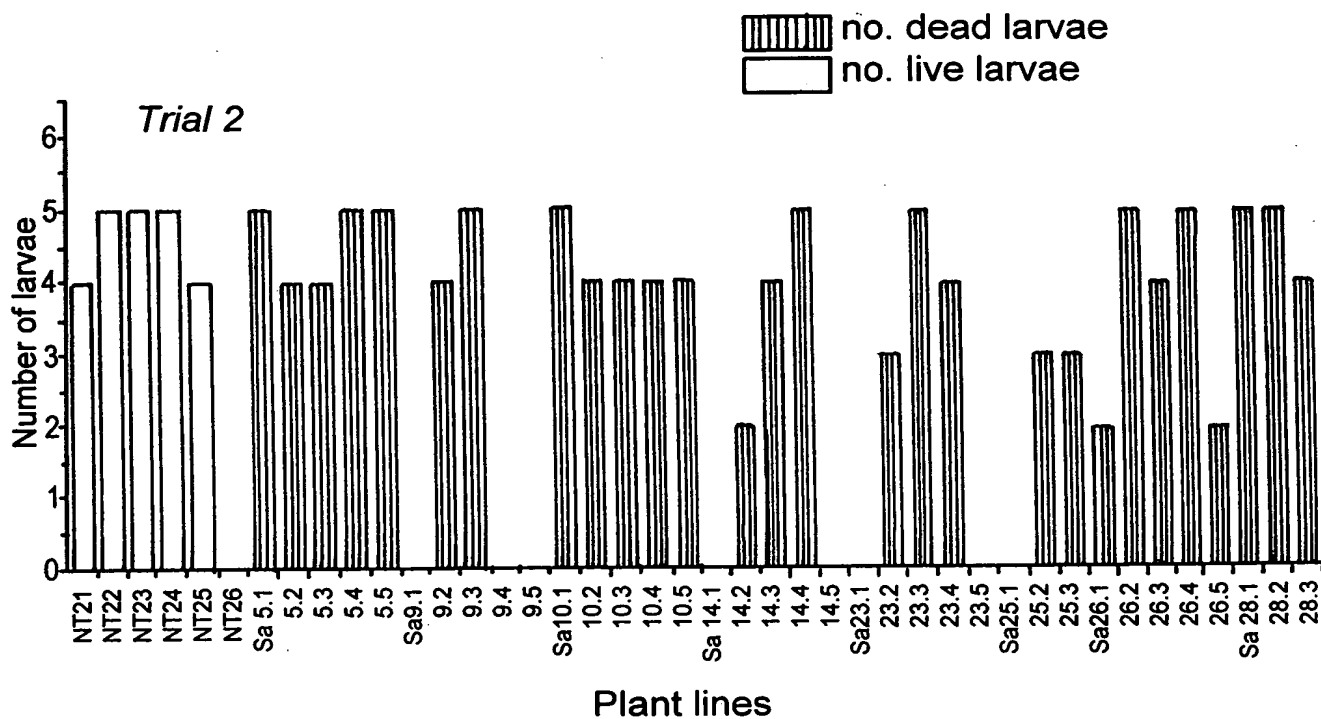
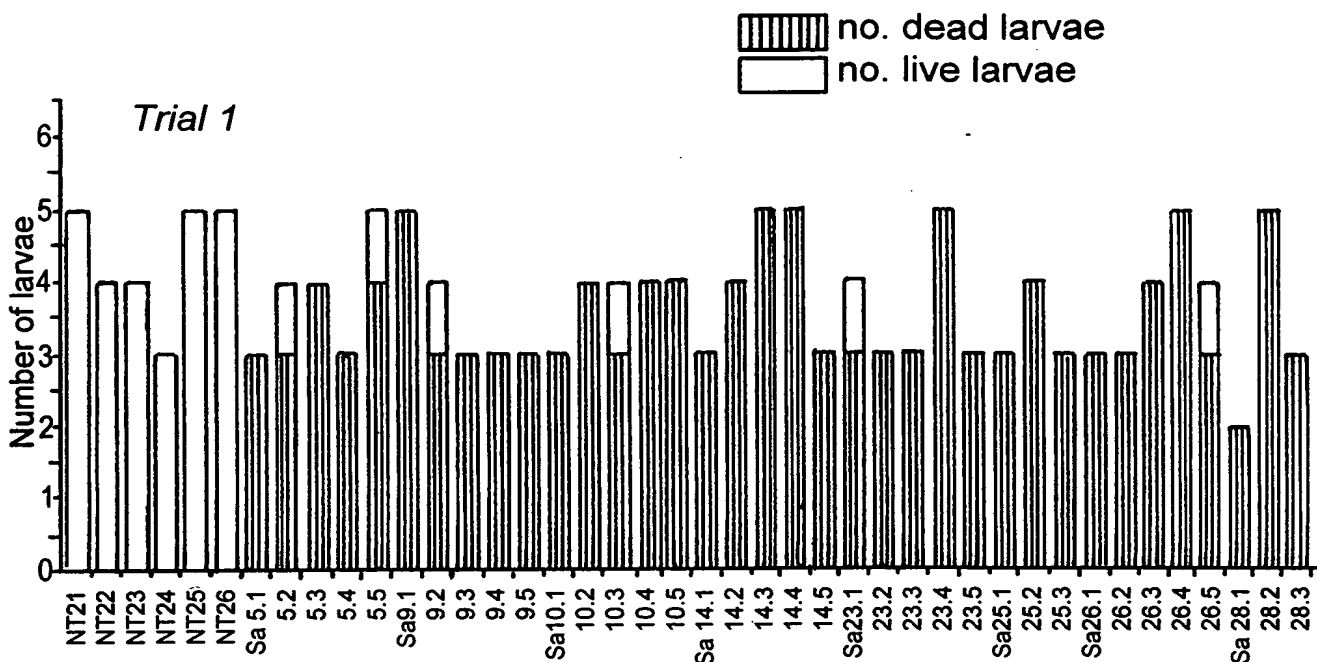


FIG. 17



12/28

+

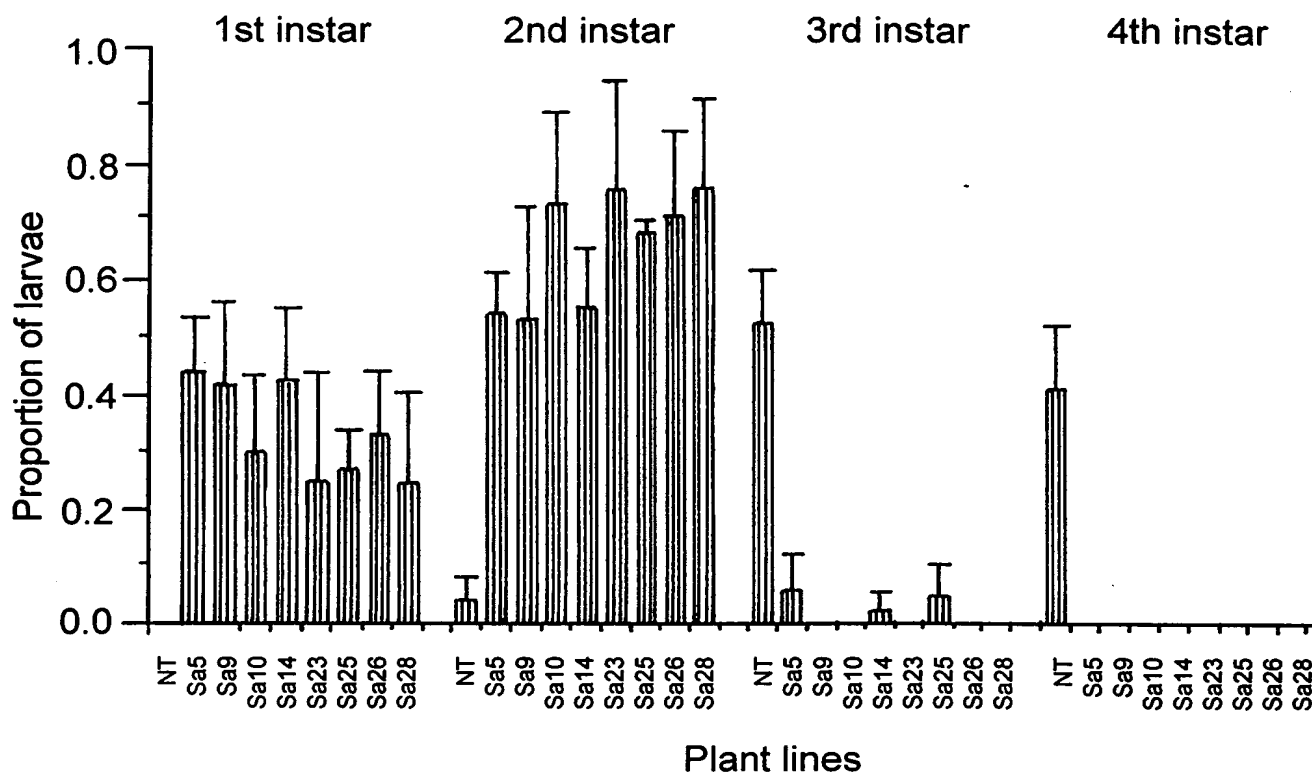


FIG. 18

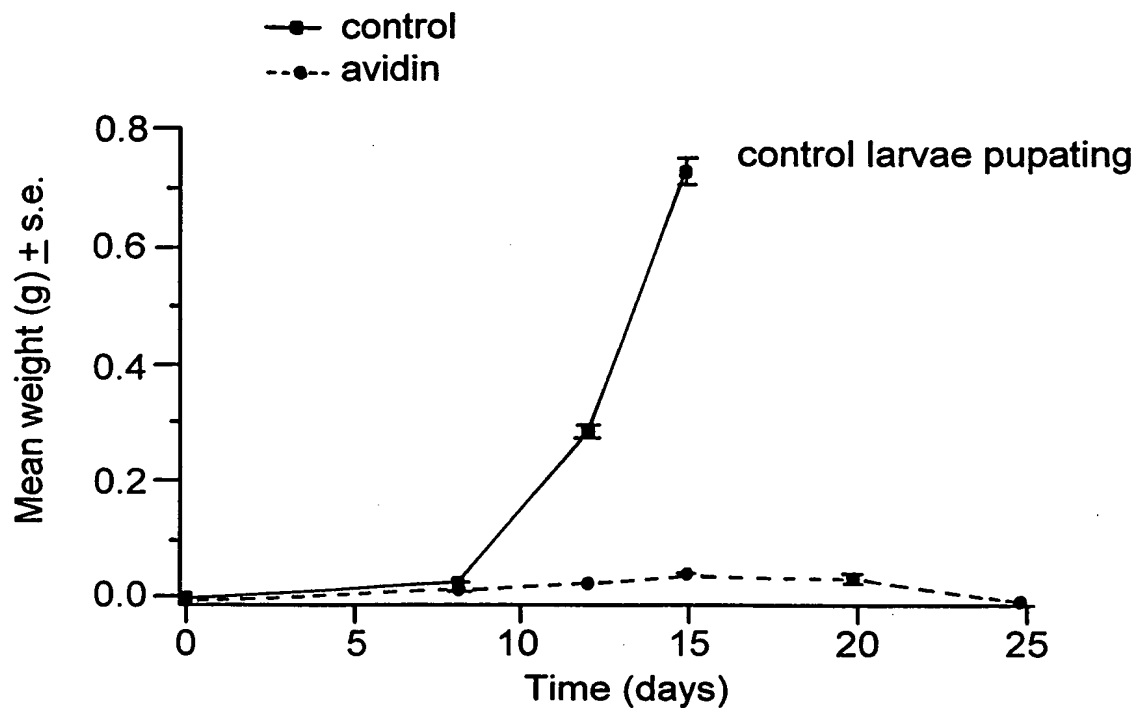


FIG. 19

+

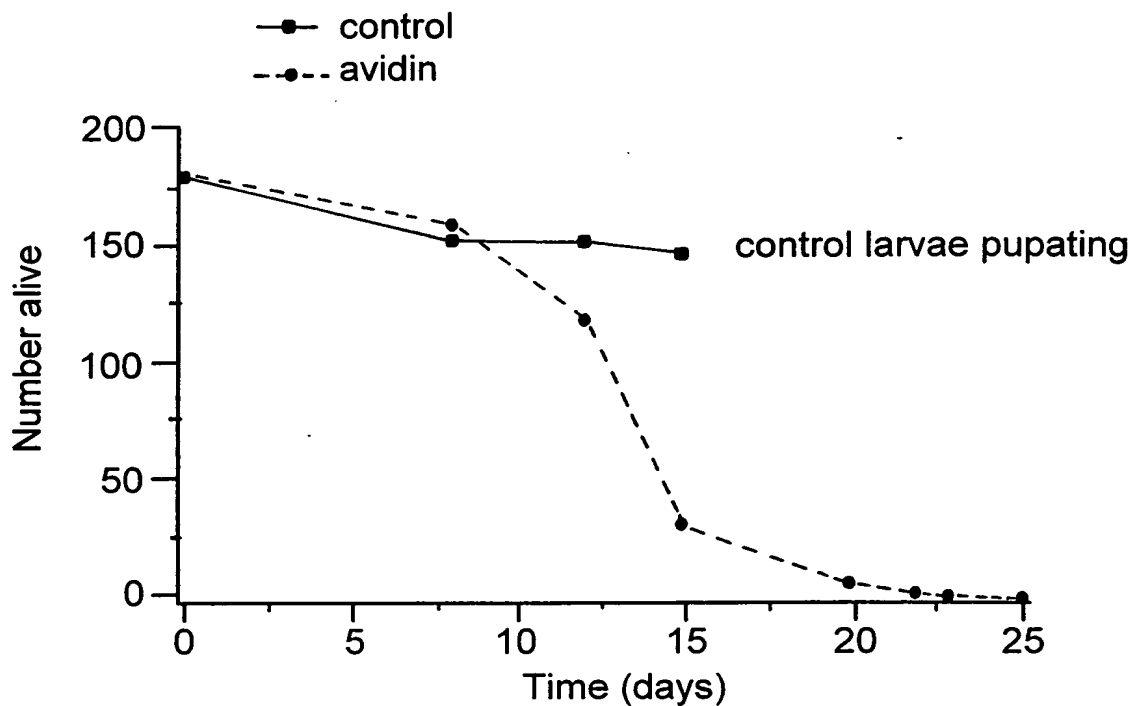


FIG. 20

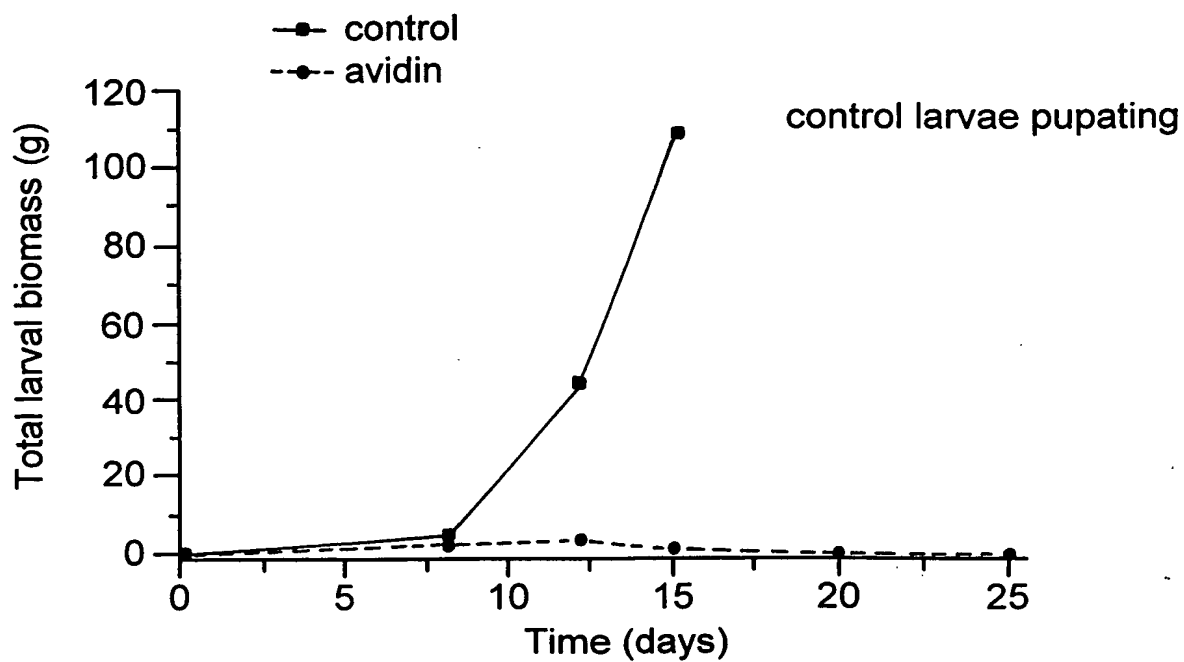


FIG. 21

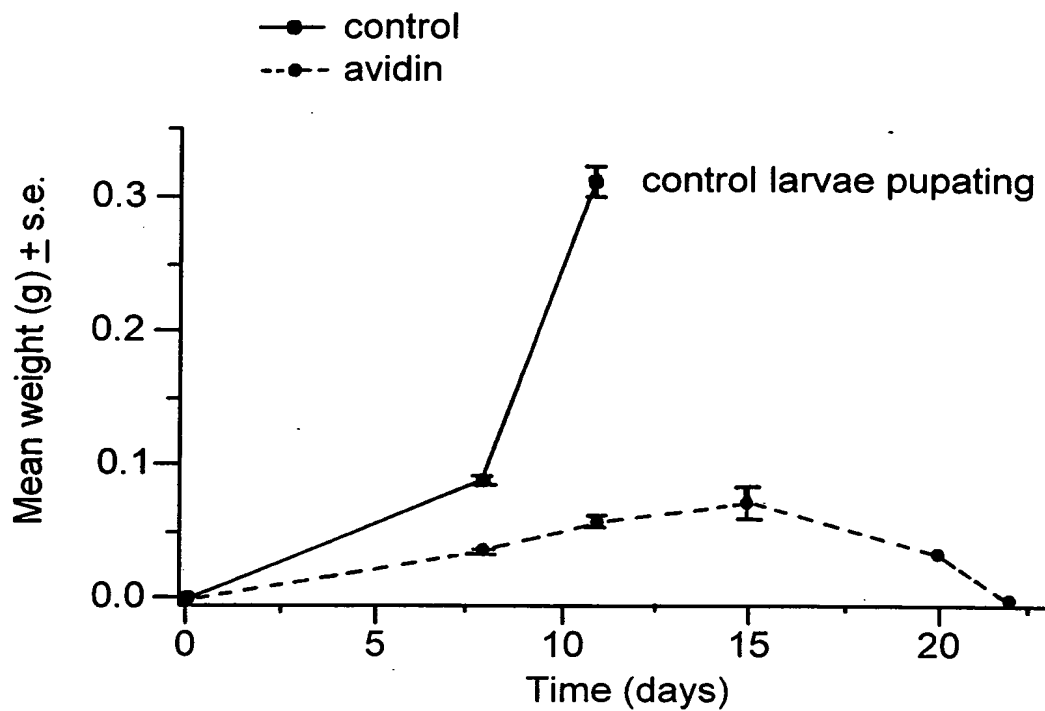


FIG. 22

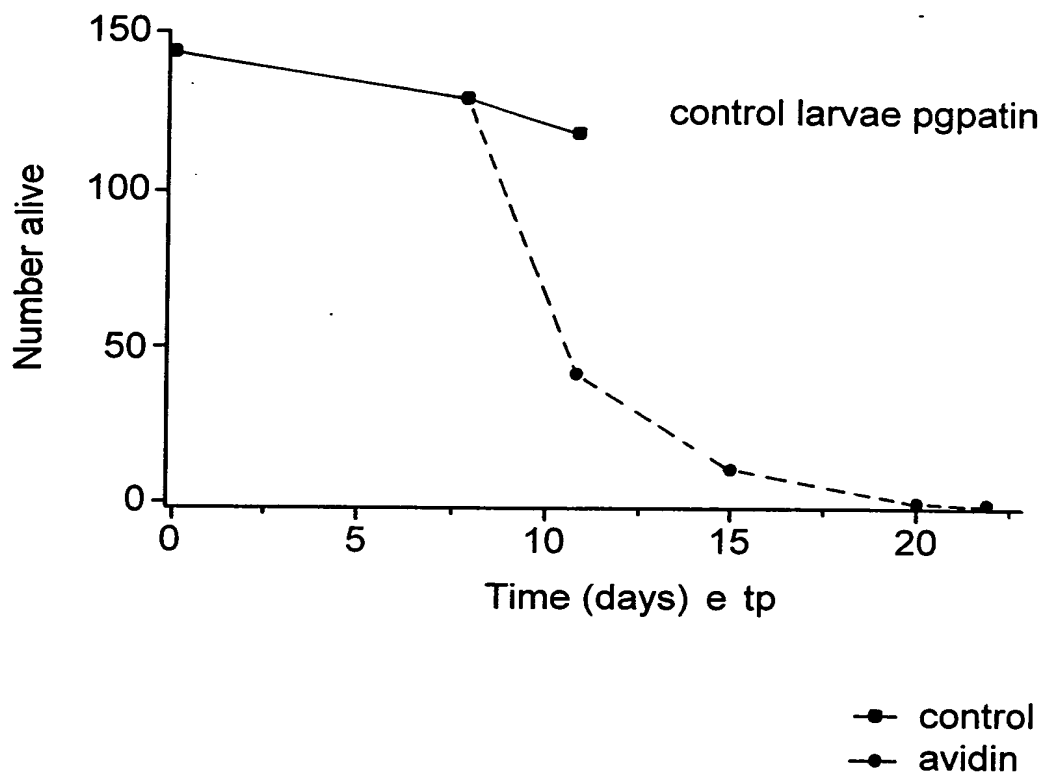


FIG. 23

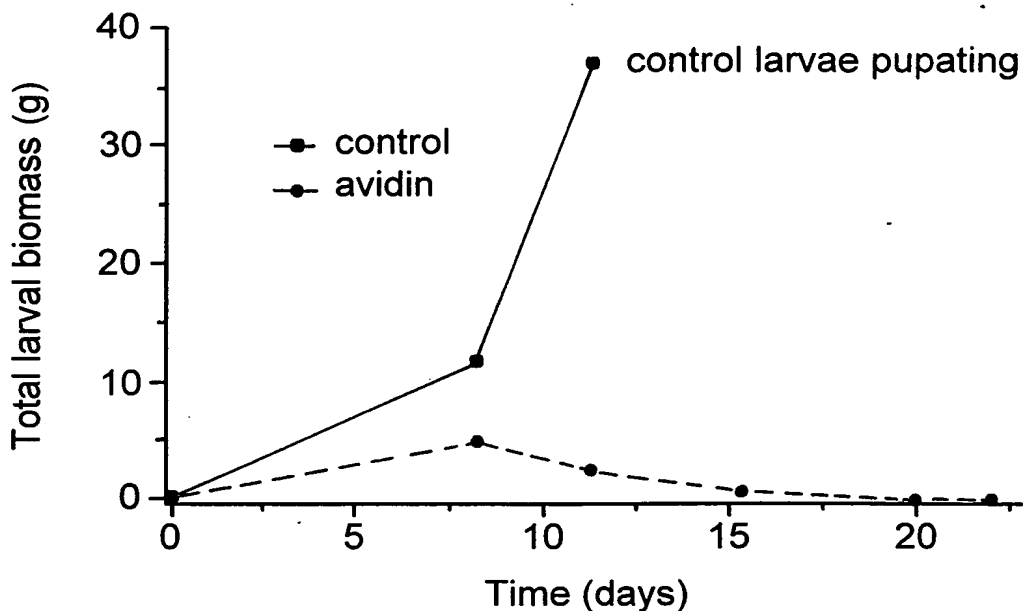


FIG. 24

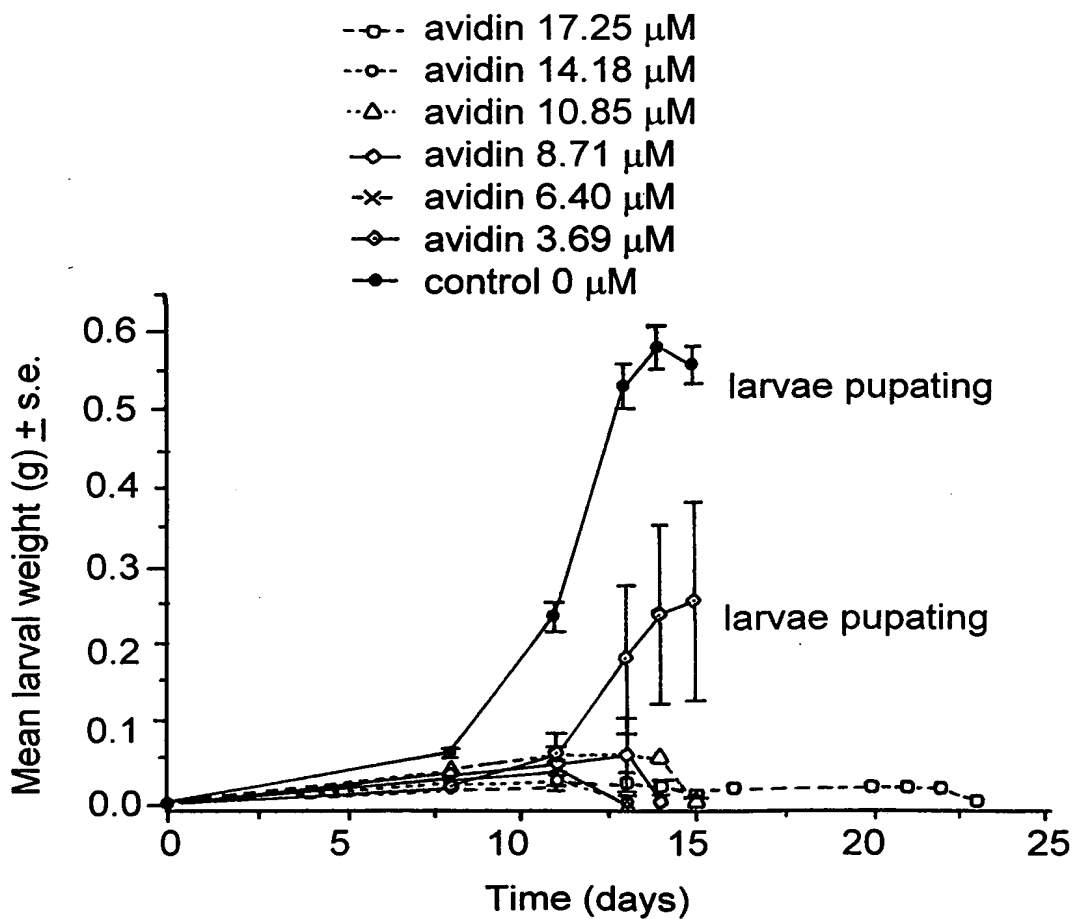


FIG. 25

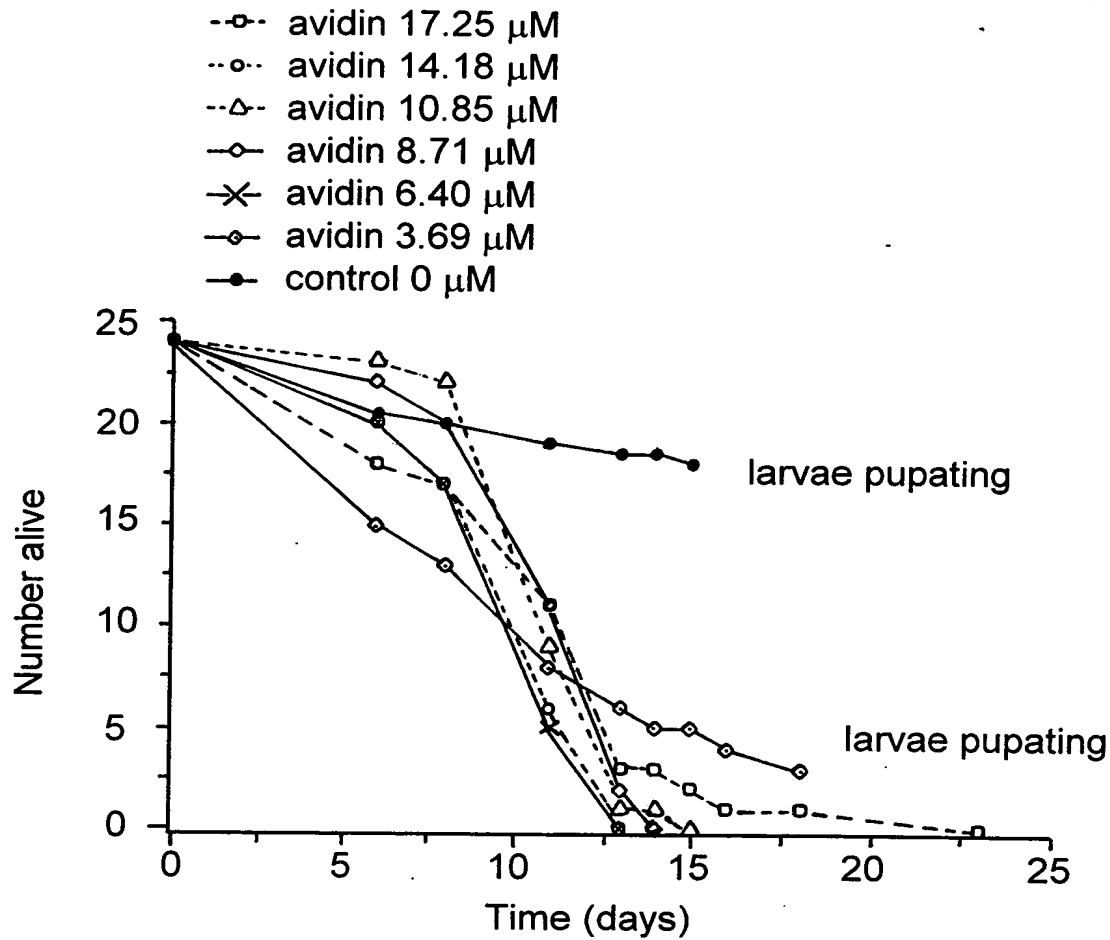


FIG. 26

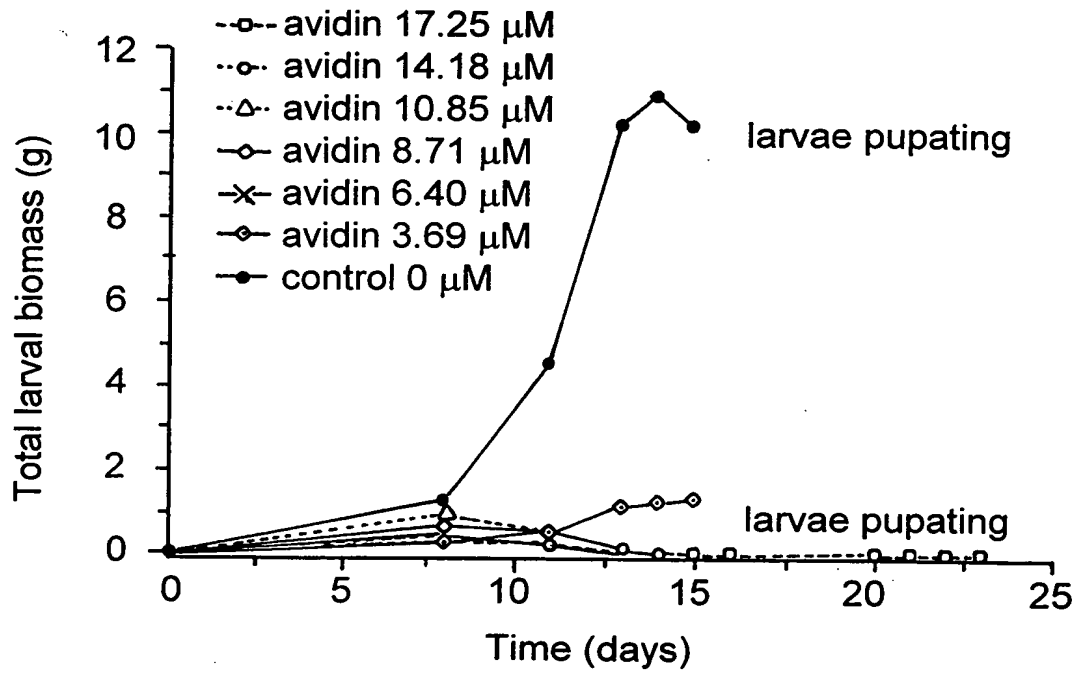


FIG. 27

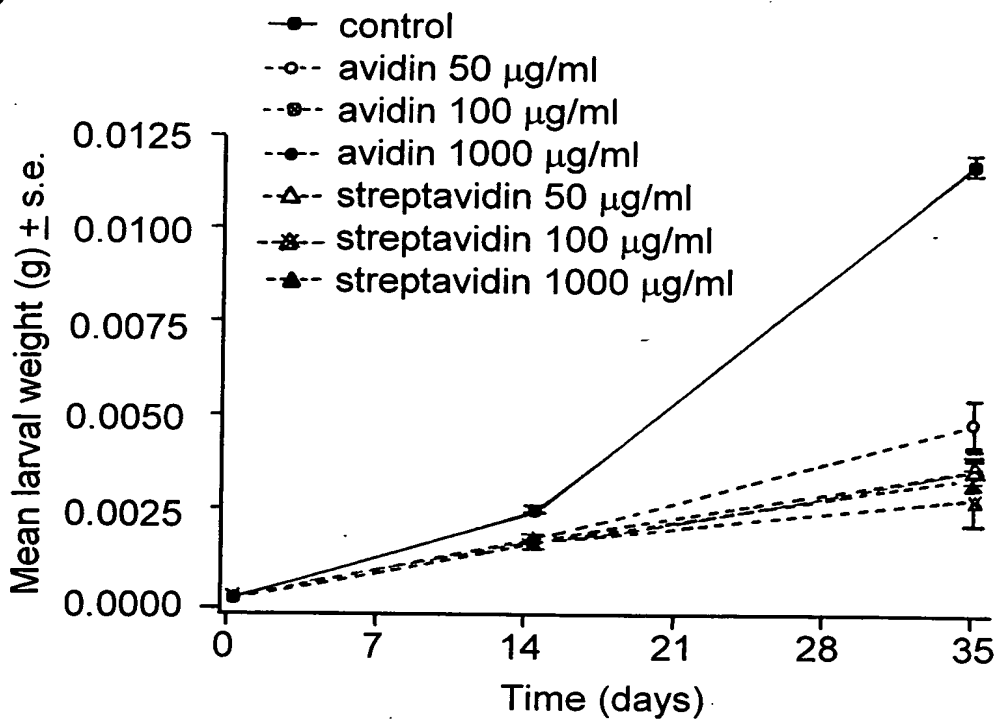


FIG. 28

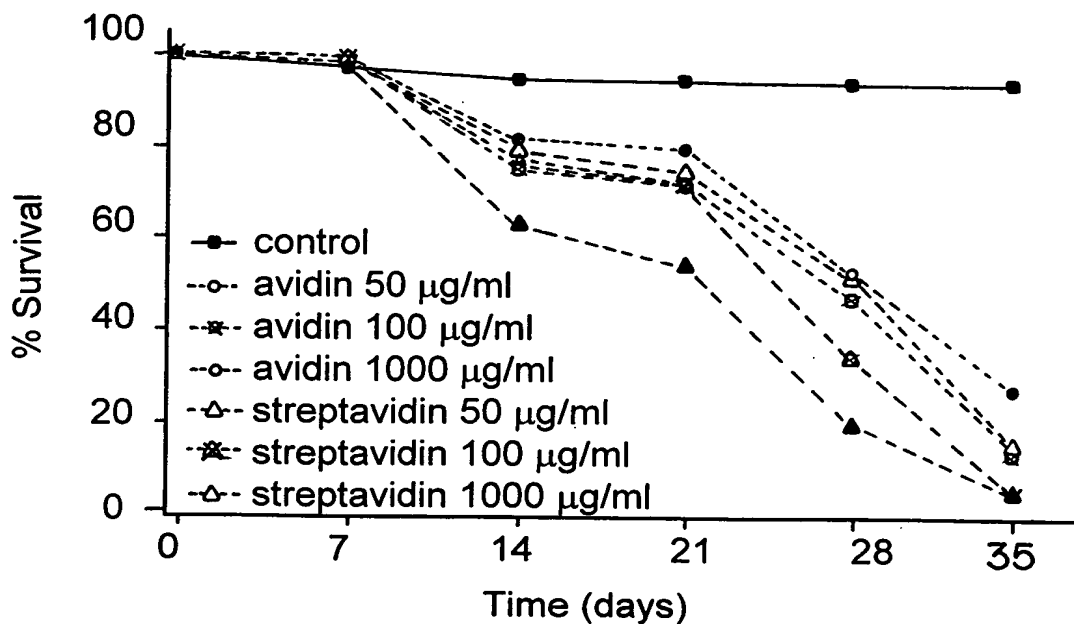
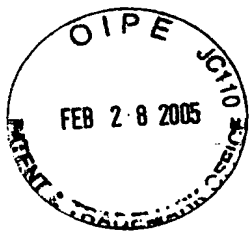


FIG. 29



19/28

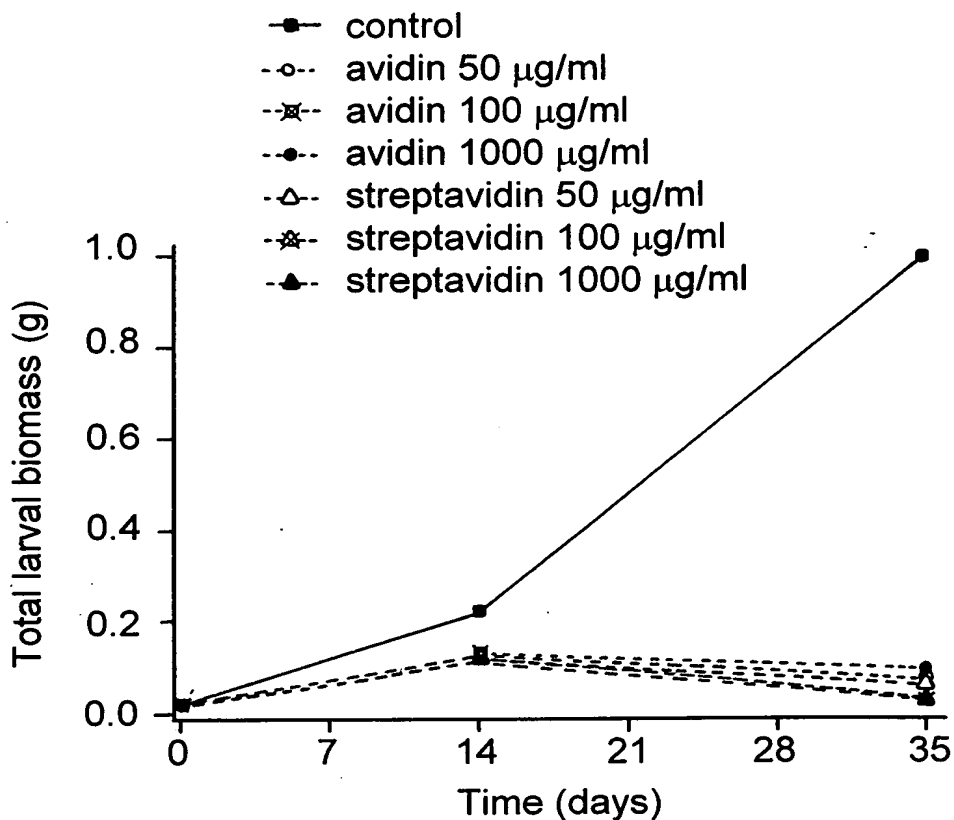


FIG. 30

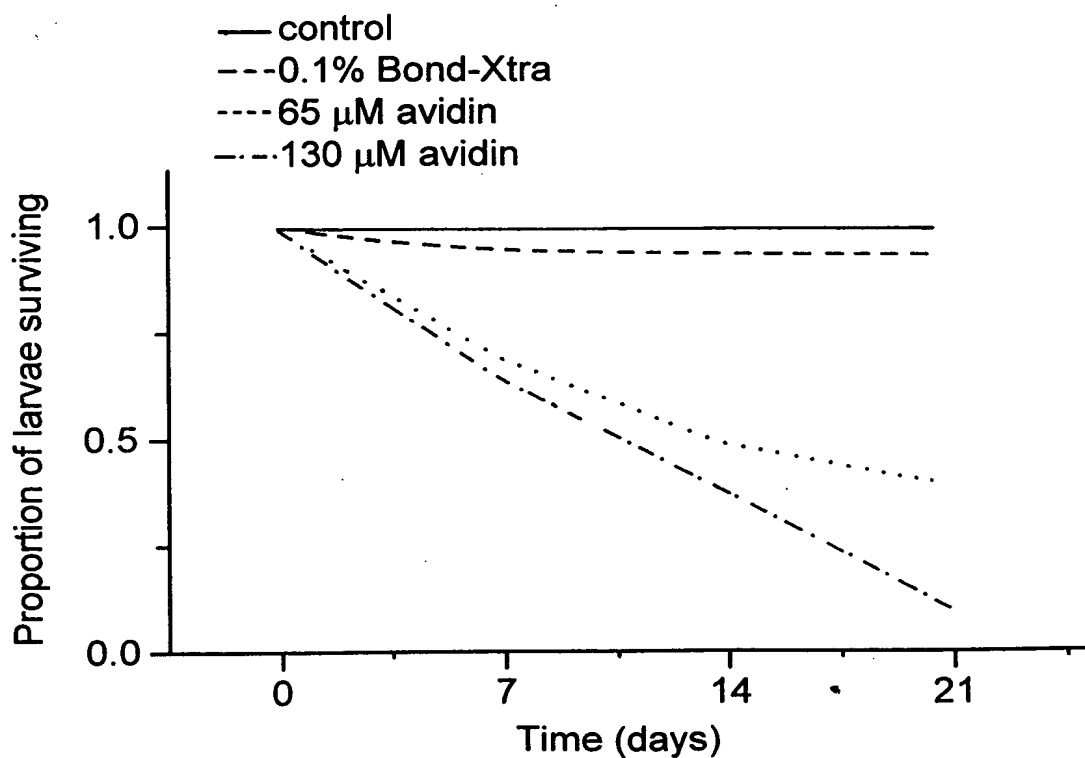


FIG. 31

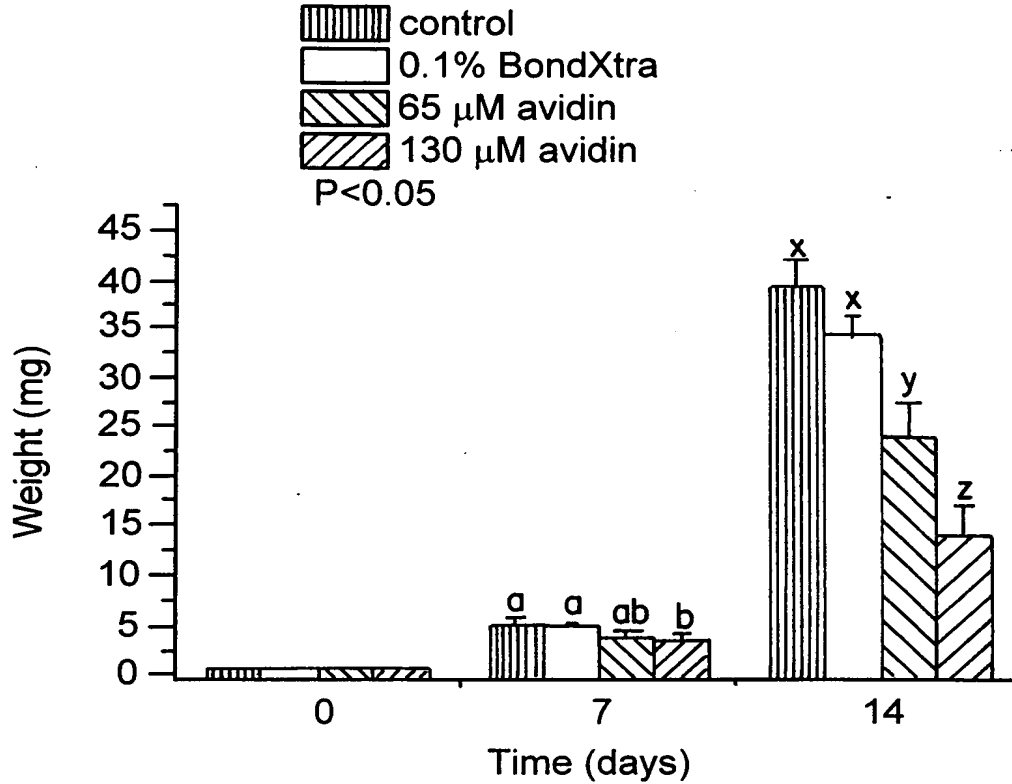
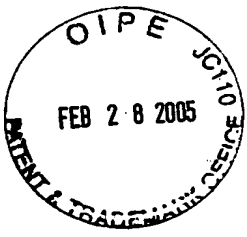


FIG. 32

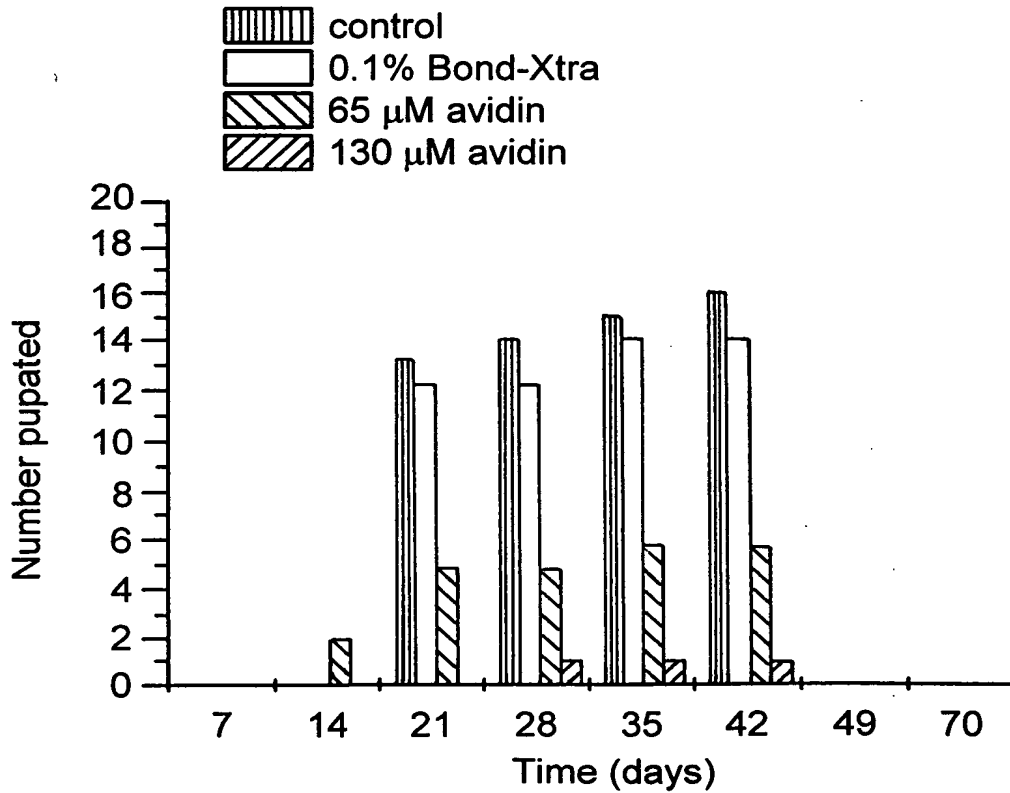


FIG. 33

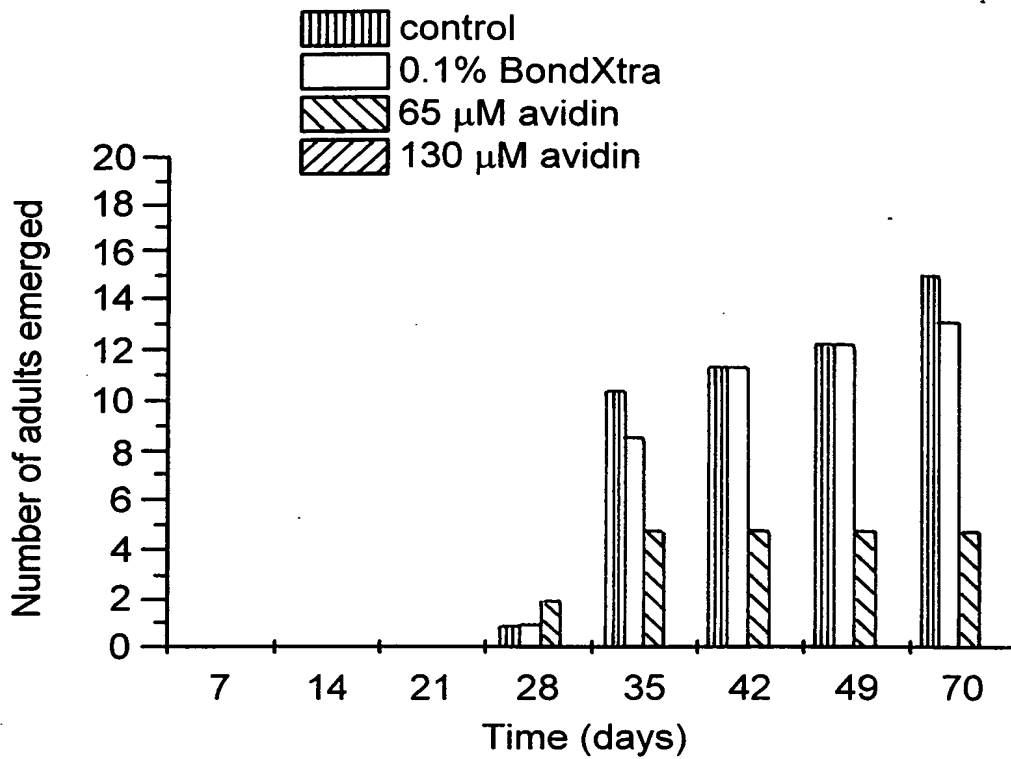
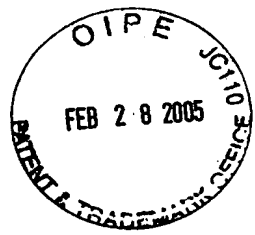


FIG. 34

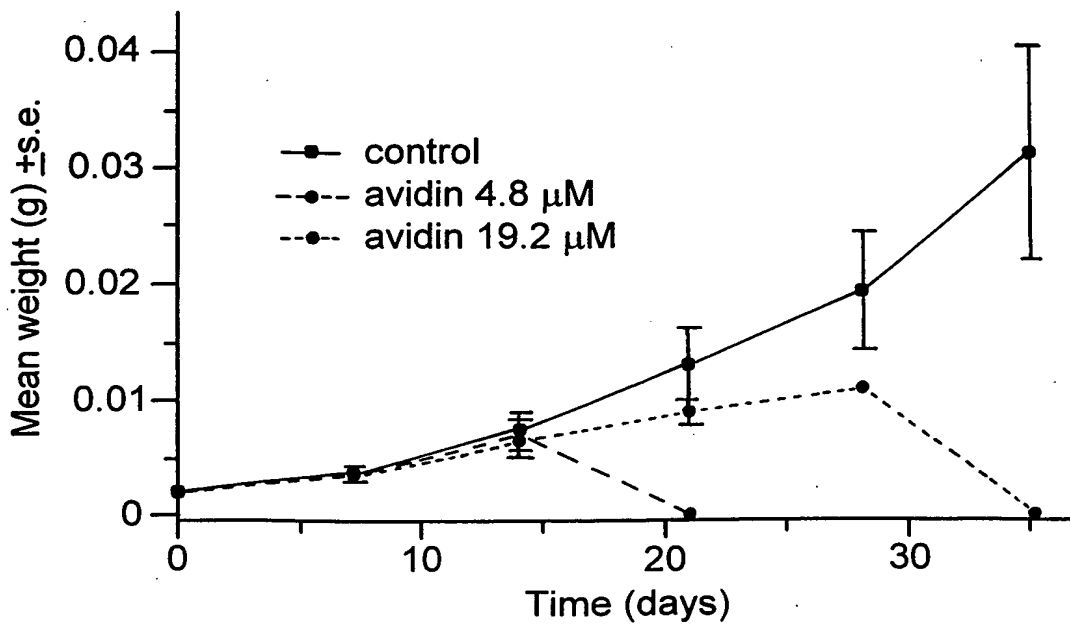


FIG. 35

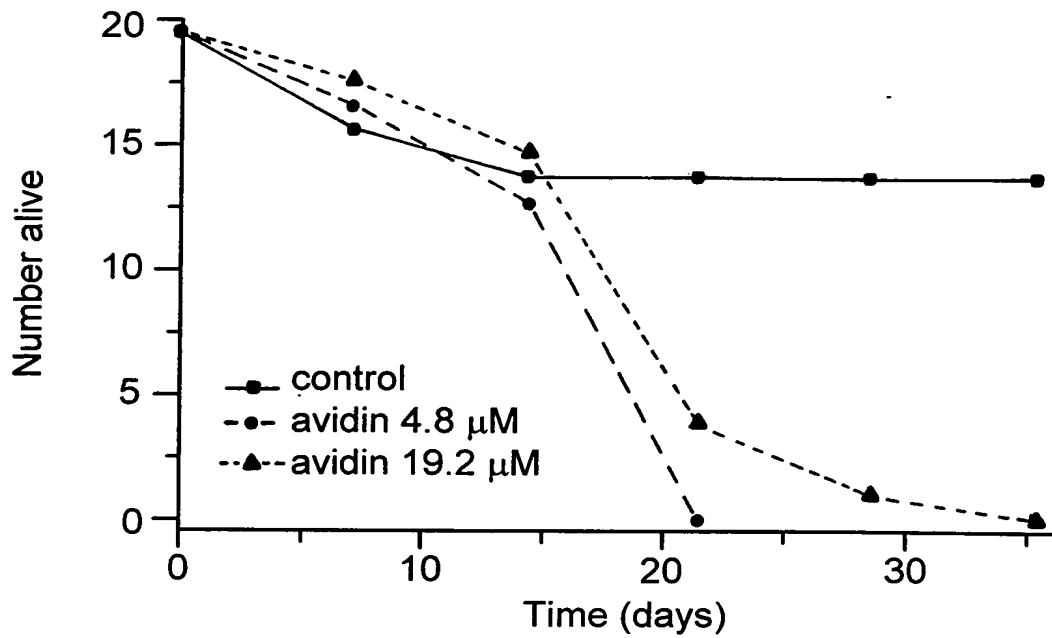


FIG. 36

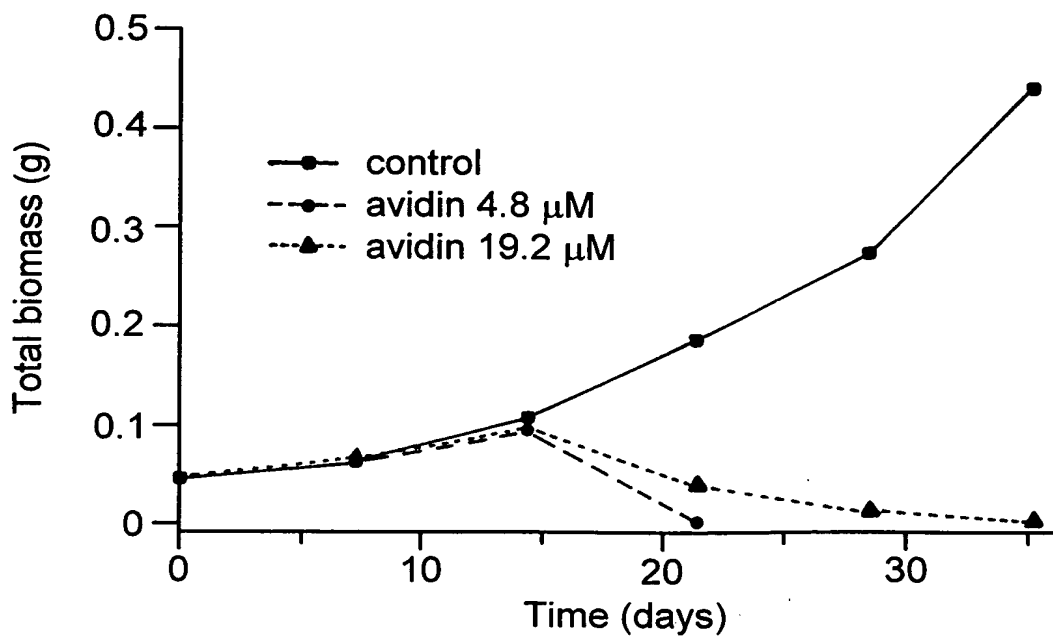


FIG. 37



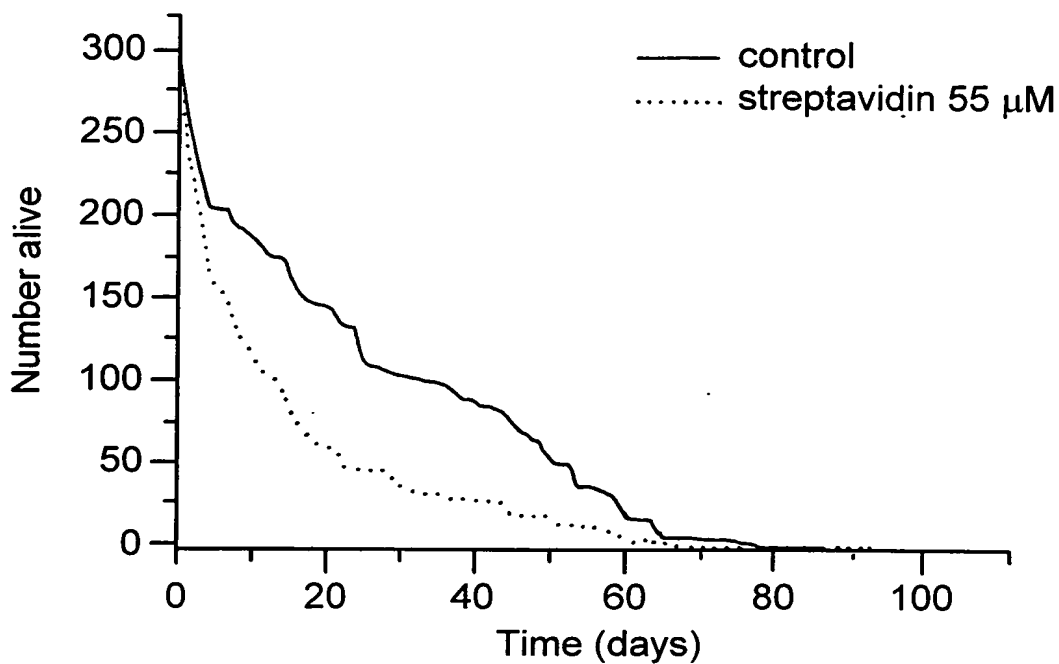


FIG. 38

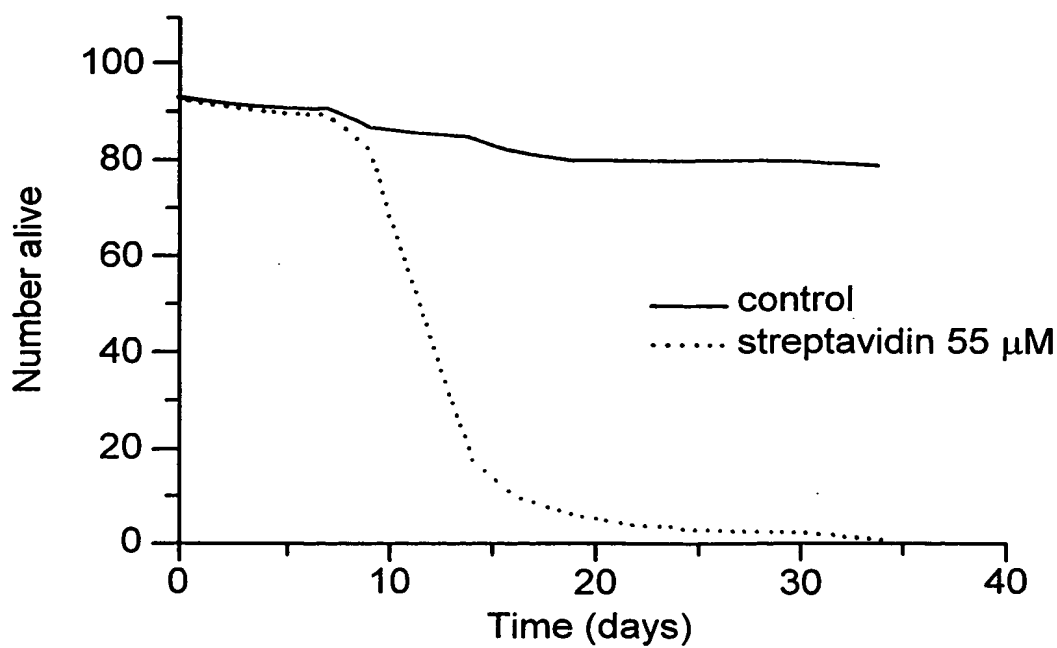


FIG. 39





+

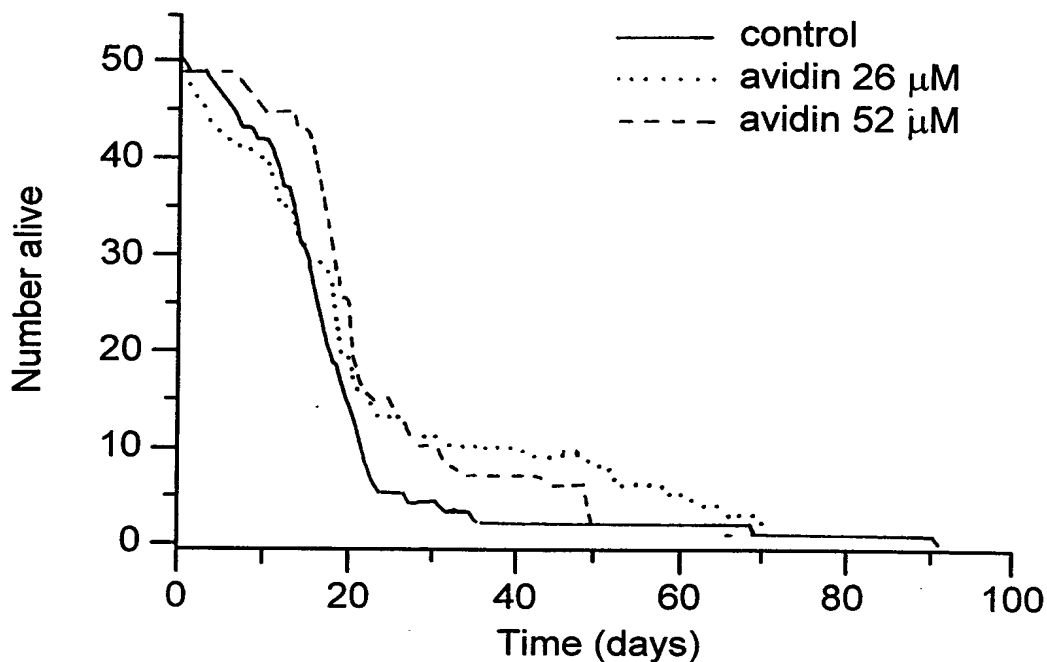


FIG. 40

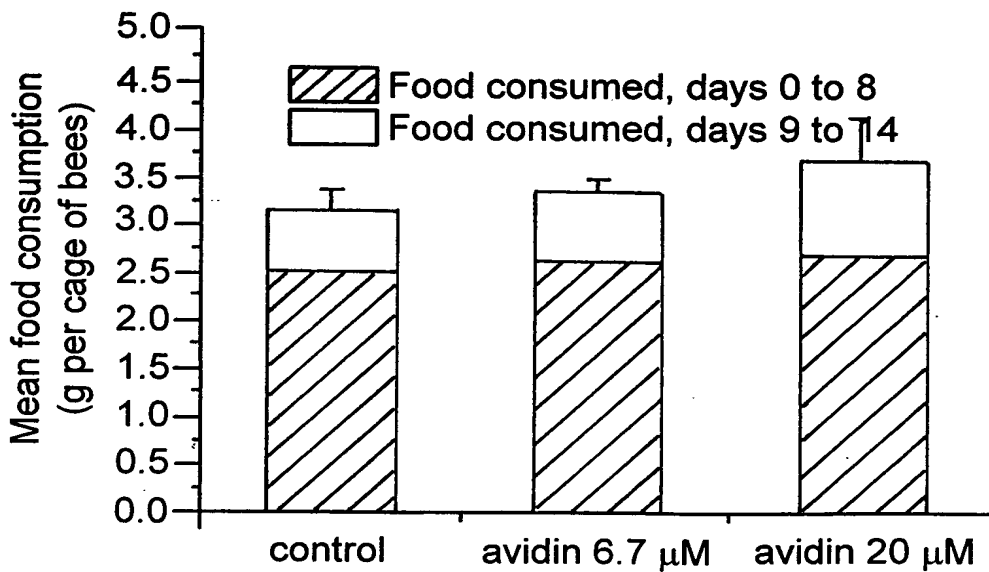


FIG. 41

+

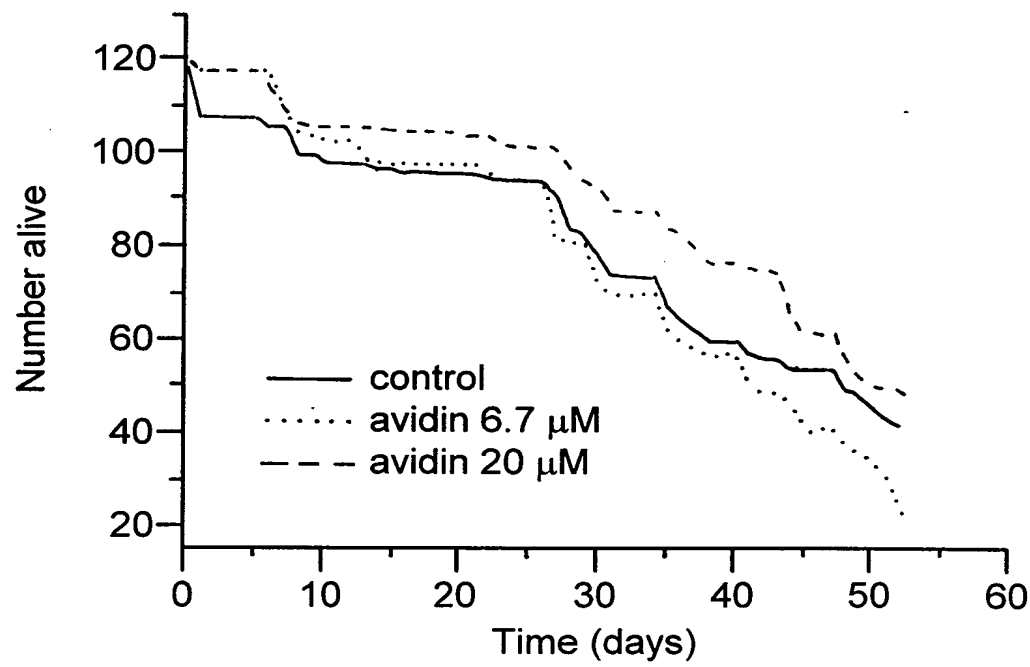


FIG. 42

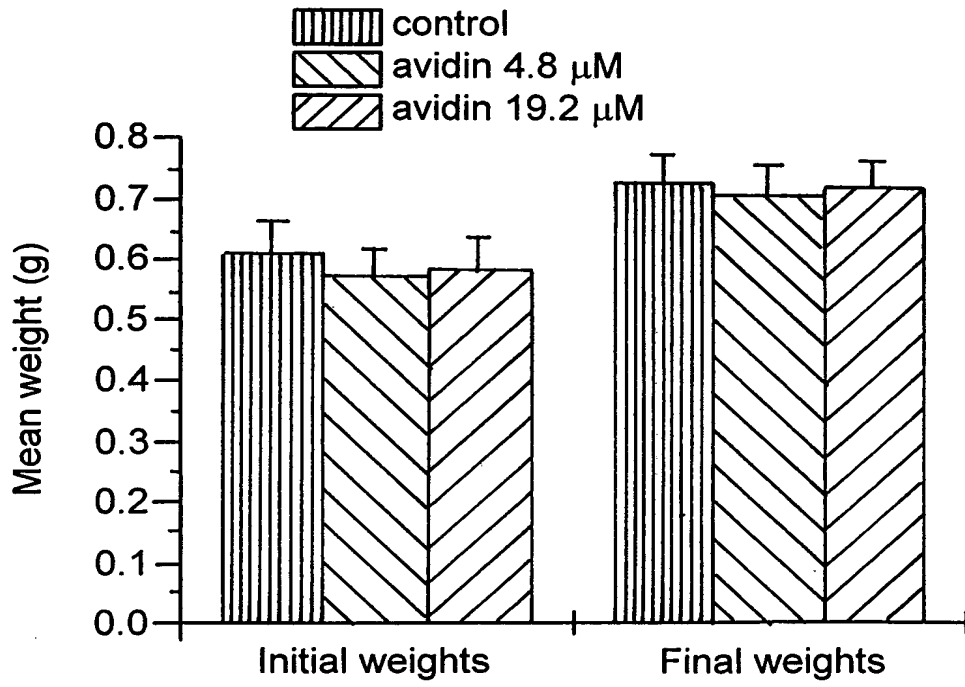


FIG. 43





+

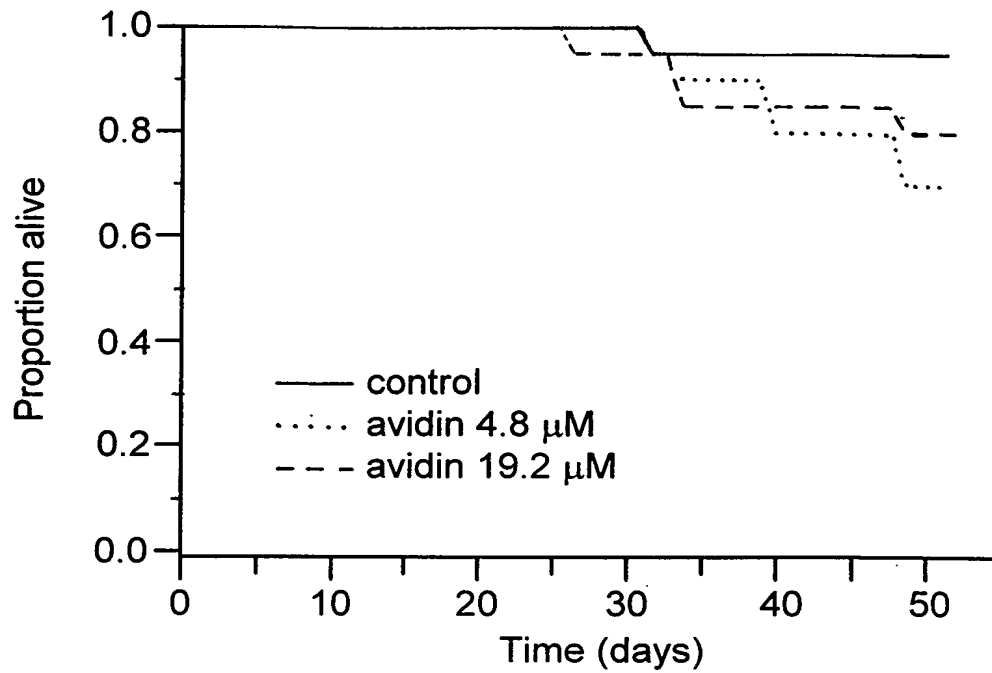


FIG. 44

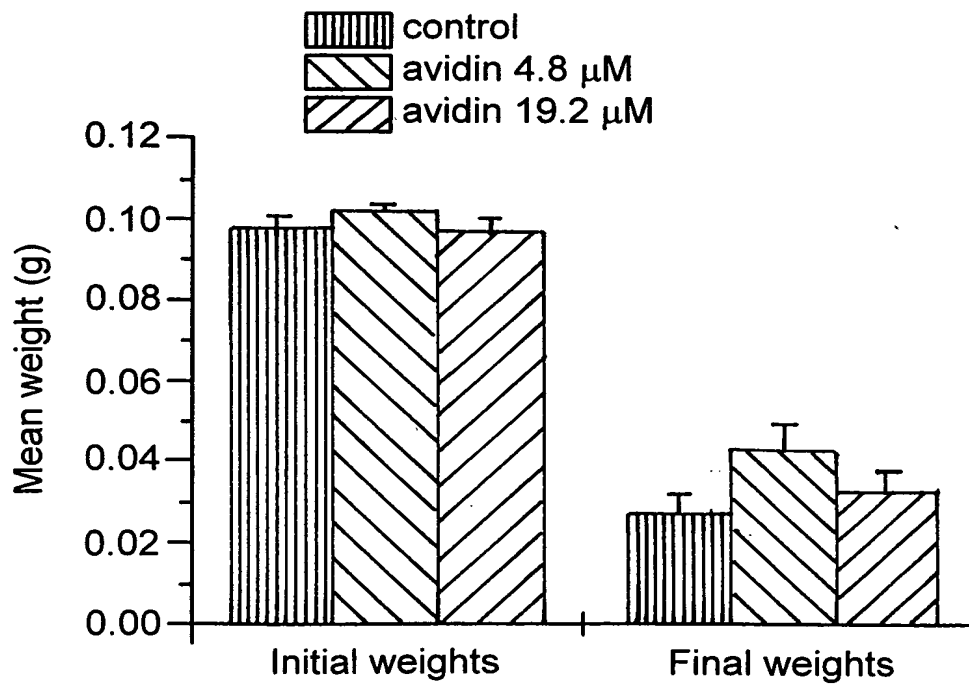


FIG. 45

+

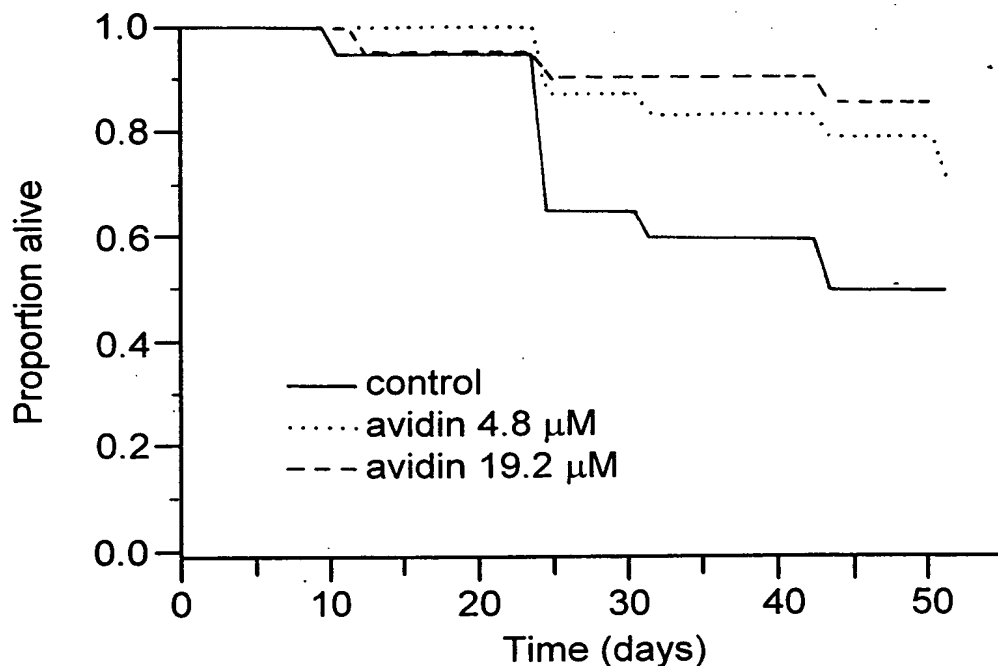


FIG. 46

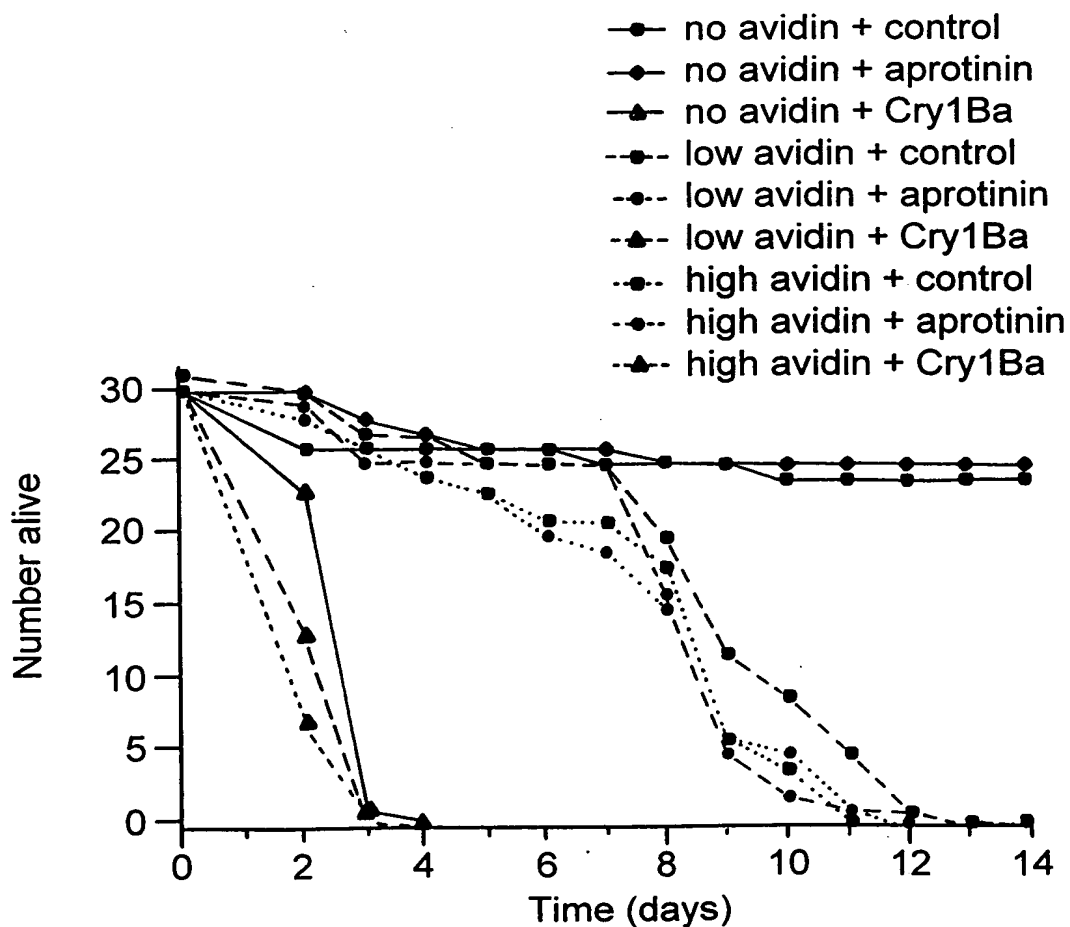


FIG. 47

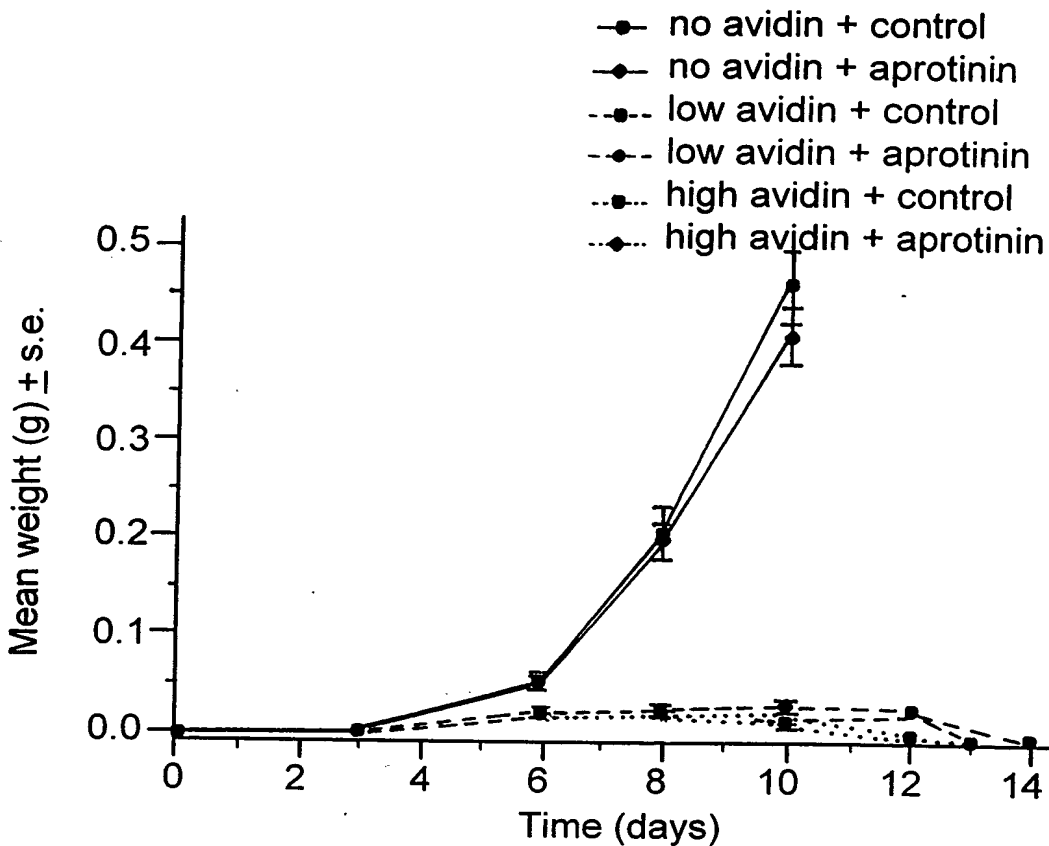


FIG. 48

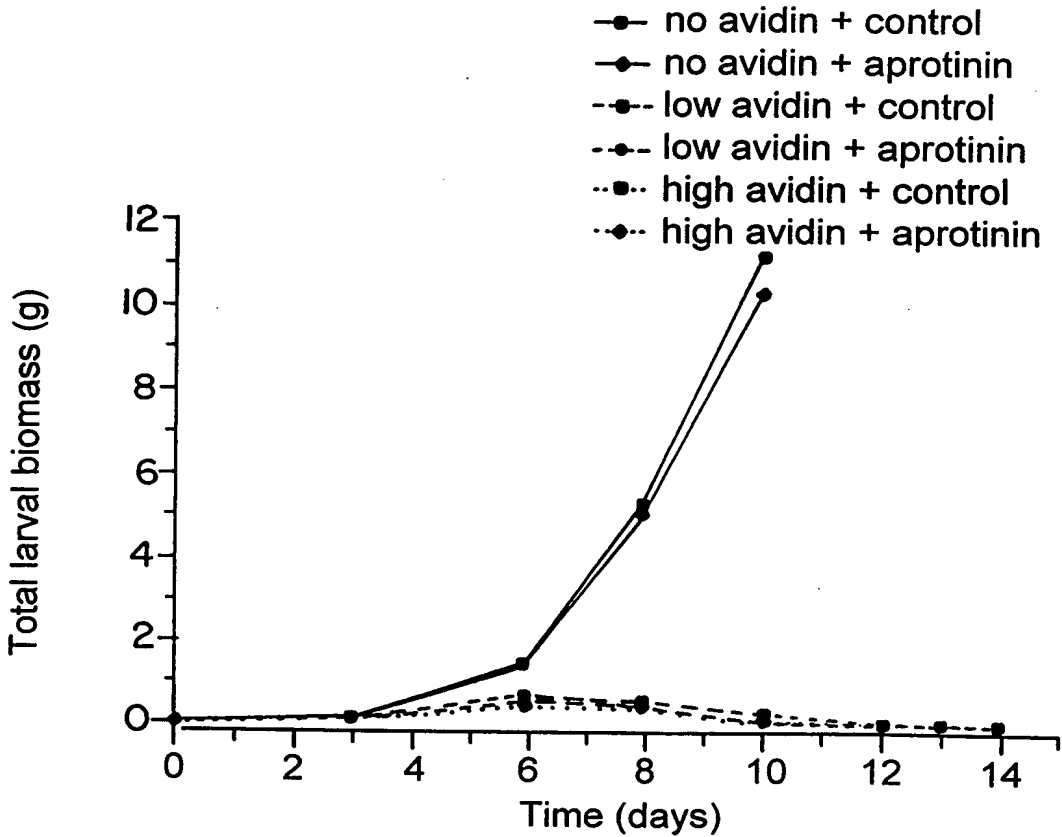


FIG. 49